序　言

農業生技產業是當今世界產業發展明星產業之一，我國農業生物技術已有良好基礎及成果，應用尖端生物技術、開發高水準生技產品，將有助於提升我國生技產業之競爭力。由國科會、農委會、中央研究院等機構共同推動之農業生物技術國家型科技計畫第三期推動規劃之十五項目標產業項目包含「中草藥及保健食品產業化體系之建立」，其中苦瓜爲經過篩選研究重點材料。由於國人在飲食文化和養生調理的觀念，對中草藥的功效和應用範疇接受程度頗高，所以國人在發展保健產品時，特別重視並選用天然的，當地的中草藥作爲原材料，正好配合現代保健產品的發展趨勢和特色，因此可以預期以苦瓜研發健康食品或其他保健產品，將有很大的發展潛力。

苦瓜在藥用上的研究，主要著重於降血糖上，而食用苦瓜具有降血糖的效果，被廣泛討論，並因其功效特殊，受到國科會國家型計畫列爲台灣中草藥及健康食品產業化體系之建立及推動之重點研究材料。行政院農業委員會花蓮區農業改良場選擇優良的地方種山苦瓜及野生苦瓜利用雜交育種技術於 2005-2007 年育成 4 個山苦瓜品種與國家型計畫相關學術研究單位合作進行保健機能方面之試驗，成果豐富，希望藉由學術相關文章的發表，增加大家對苦瓜多樣性功效的認識，進而發展形成苦瓜之保健產業。本次研討會邀請參與國科會國家型計畫苦瓜研究之專家學者，就台灣中草藥及健康食品產業化體系之建立及推動、苦瓜降血糖與生物活性等研究發表 9 篇論文，將目前苦瓜研究產業發展藥用保健功能之開發成果介紹給國人，藉由各界廣泛深入之交流討論，促進未來苦瓜保健產業之發展，並藉由研討會之召開結合國內醫藥界與農業研發團隊建立良好苦瓜栽培生產模式，以高品質苦瓜原料，建立良好之苦瓜保健產業，並提昇台灣在全球化保健產品產業市場之競爭力。

本次研討會承蒙各界人士及專家學者的熱心協助，使研討會之籌備工作及研討會之進行一切順利成功，深表感謝。本論文專刊於編印期間，承每位作者充分配合，乃能順利付梓，惟本刊涵蓋內容甚廣，付印匆促，疏忽難免，期不吝指正。本論文專輯係凝聚各方之智慧，期盼能擴展研討會成果，使對苦瓜及其保健功效有興趣之民眾及相關單位獲得更多的相關資訊，增加國人愛用苦瓜，以促進苦瓜相關產業升級農民收益。

行政院農業委員會花蓮區農業改良場

場長　侯福分　謹識

中華民國九十七年六月

Herb-based Functional Foods from Laboratory to The Market

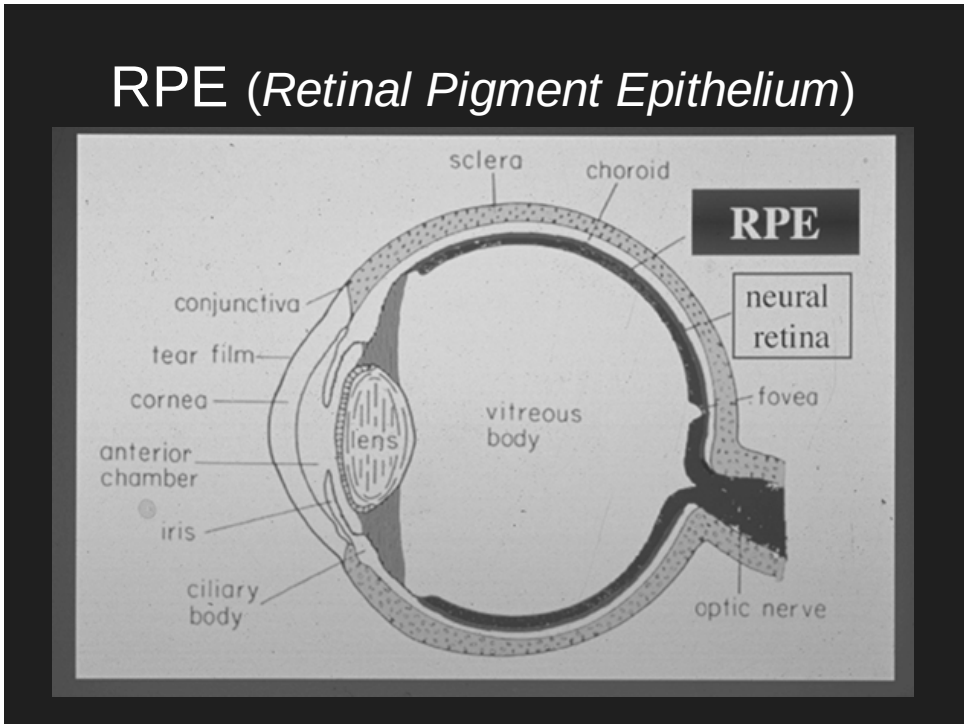
H.S. Tsay

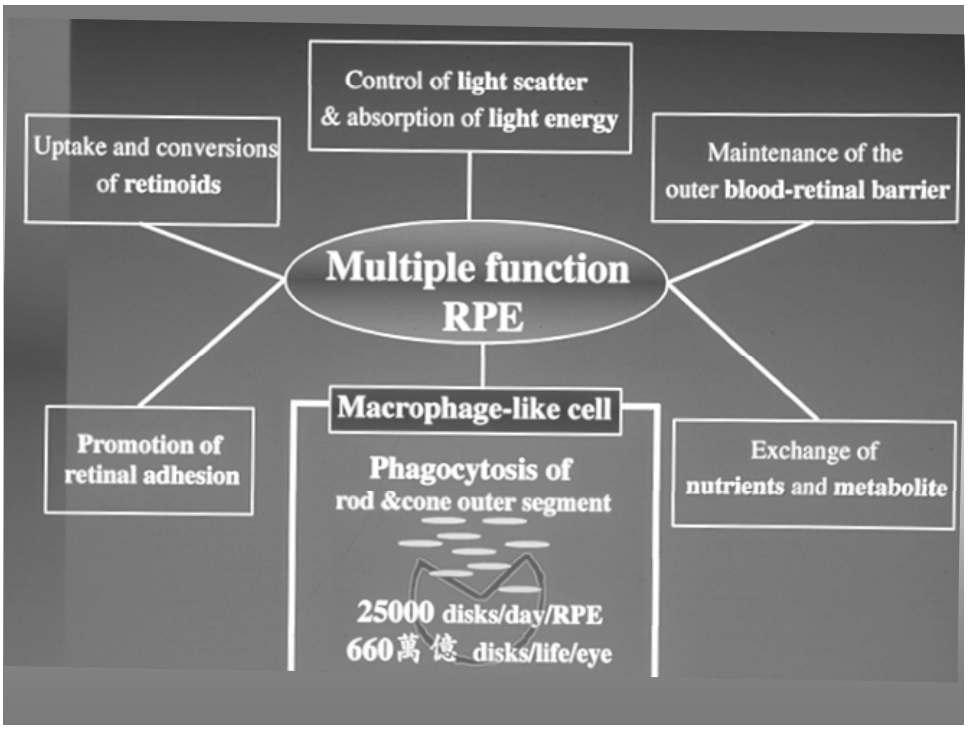
National Science and Technology Program for Agricultural Biotechnology
Council of Agriculture, National Science Council,
Chaoyang University of Technology
TAIWAN

Commercial Application of Research Projects

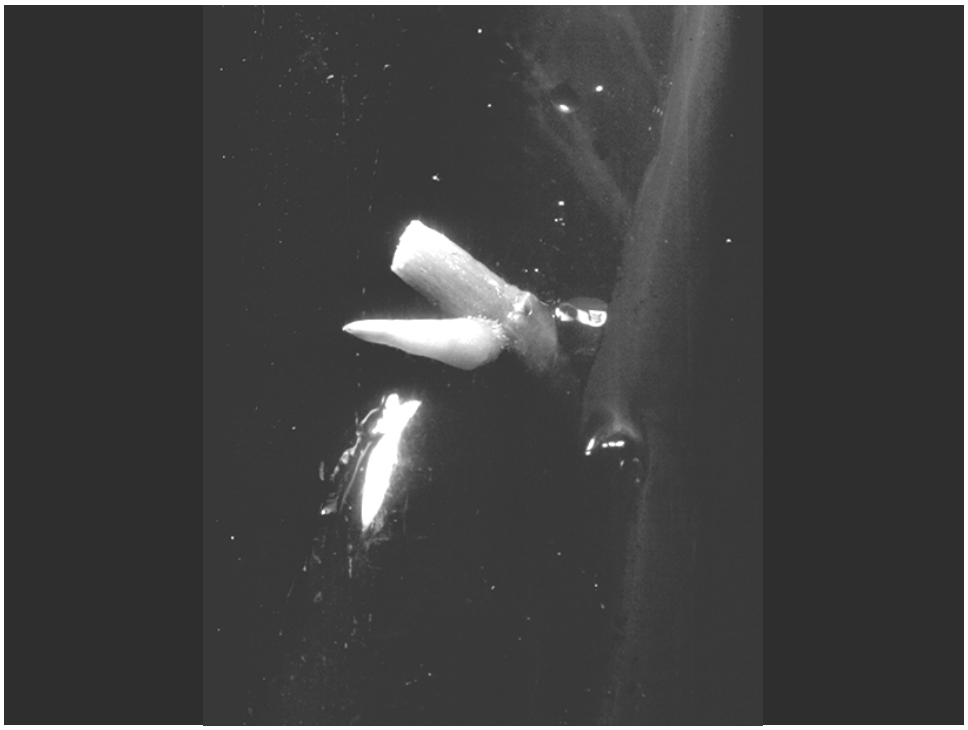
1. *Dendrobium huoshanense*（Yang-Ming University）
2. *Bupleurum Kaoi*（Kao-Hsiung Medical University and Taiwan Agricultural Research Institute ）
3. *Cordyceps sinensis*（Taiwan Sugar Company）
4. *Anoectochilus formosanus*（China Medical University）
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9. *Antrodia cinnamomea*（Chung-Hsing University）
10. Coconut（Yang-Ming University）


HUANG
霍山石斛

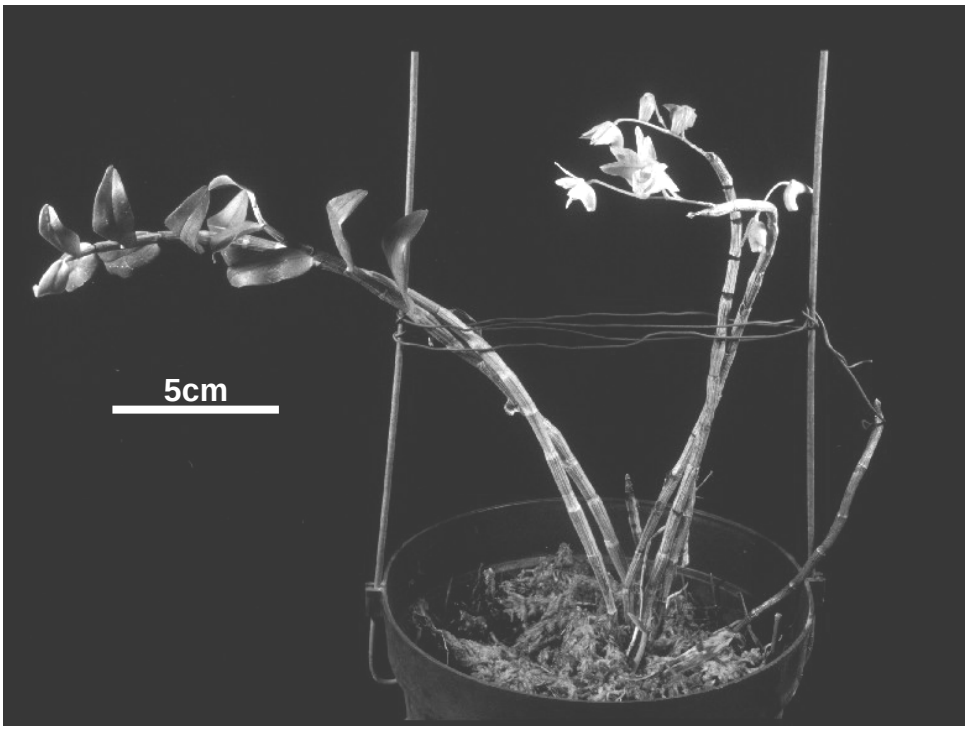

RPE (Retinal Pigment Epithelium)
sclera
choroid
RPE
neural retina
conjunctiva
tear film
cornea
anterior chamber
iris
ciliary body
lens
vitreous body
fovea
optic nerve


Control of light scatter
& absorption of light energy
Uptake and conversions
of retinoids
Maintenance of the
outer blood-retinal barrier
Multiple function
RPE
Macrophage-like cell
Promotion of
retinal adhesion
Phagocytosis of
rod &cone outer segment
Exchange of
nutrients and metabolite
25000 disks/day/RPE
660萬億 disks/life/eye


2cm



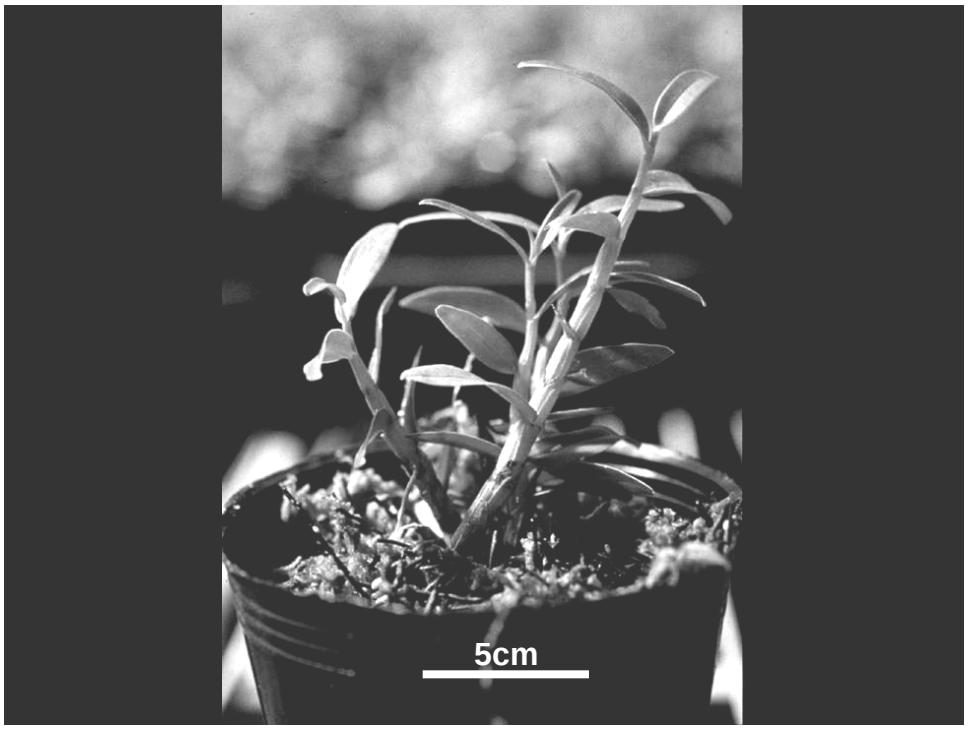


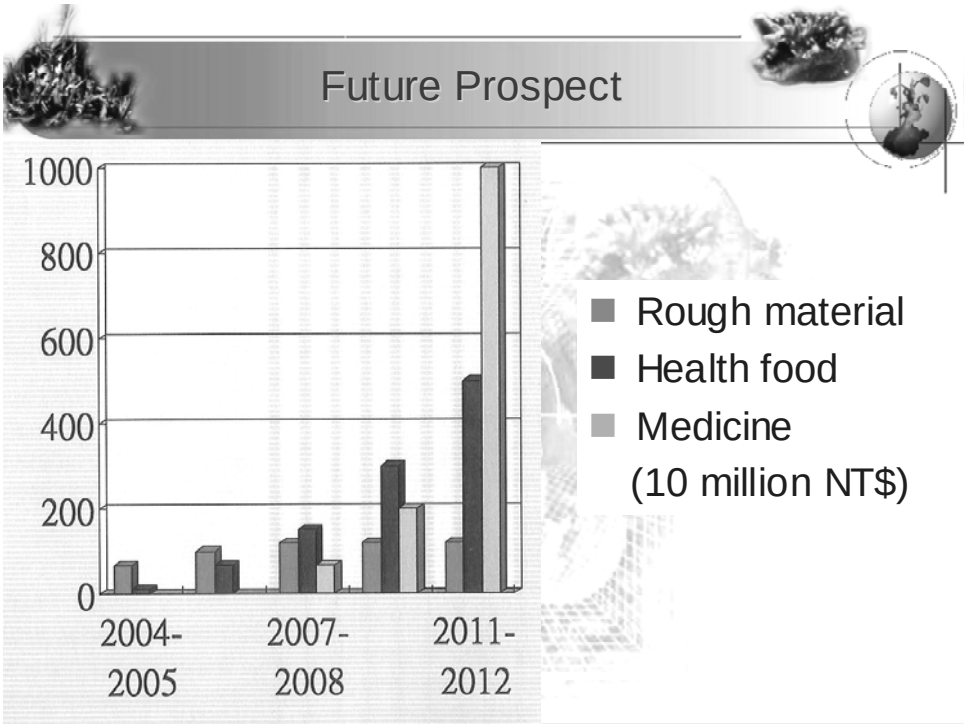

5cm








5cm


Future Prospect
1000
800
600
400
200
0
2004-2005
2007-2008
2011-2012
Rough material
Health food
Medicine
(10 million NT$)


永豐堂
霍山石斛


霍山石斛
永豐堂
霍山石斛

Commercial Application of Research Projects

1. *Dendrobium huoshanense*（Yang-Ming University）
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10. Coconut（Yang-Ming University）



Bupleurum kaoi Liu, Chao & Chuang

- **Family** : Umbelliferae
- **Distribute** : Endemic in Taiwan
- **Part Used** : Roots
- **Type of Plant**

  A perennial erect glabrous herb, about 40 – 70 cm high.



The roots, flowers and fruits of *B. kaoi*

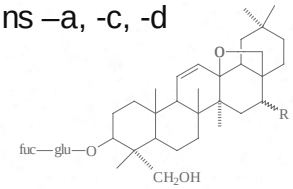
- **Medicinal Use**

  In Taiwan the water decoctions of *B. kaoi* roots has become famous as a **hepatoprotective agents**.

Bupleurum kaoi Liu, Chao & Chuang

- **Major Active Chemicals** : Saikosaponins –a, -c, -d

  The root of *B. kaoi* contains the highest amount of saikosaponins more than *B. chinense*, a species in China, and *B. falcatum*, a species in Japan.



- **Documented by Research**

  ※ The Hepatoprotective Effects of *B. kaoi* Against CCl_4-induced, or Galactosamine-induced Acute Hepatic Damage in Rats.

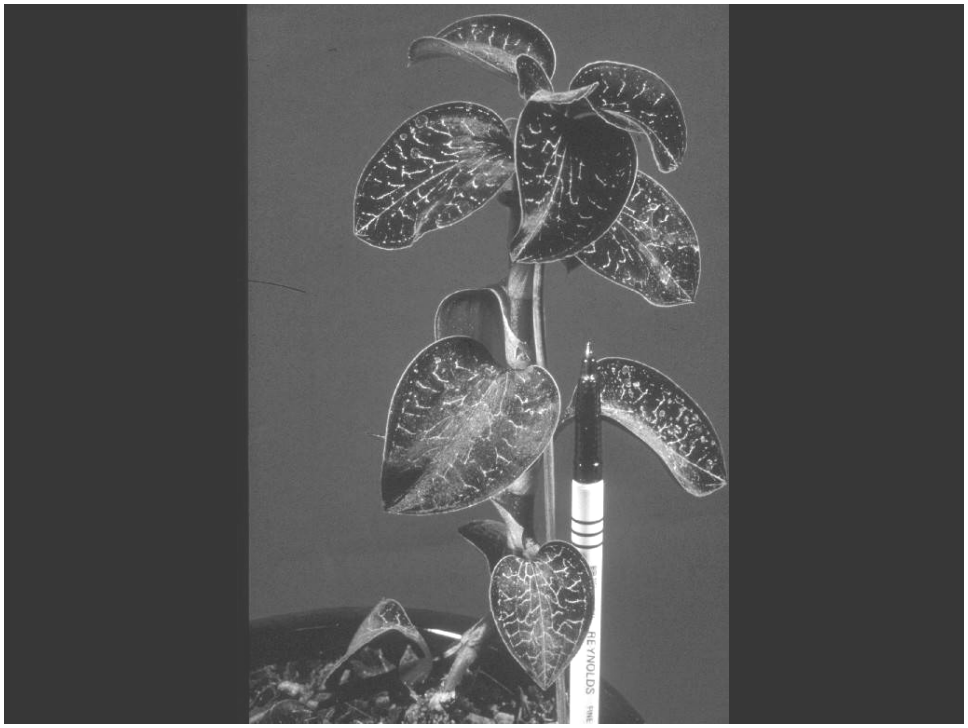
  Am. J. Chin. Med. 18：105 – 112, 1990.
  J. Ethnopharm. 34：155-165, 1991.
  Fitoterapia 55：409-417, 1994.

  ※ The Hepatoprotective Effect of *Bupleurum Kaoi*, an Endemic Plant to Taiwan, Against Dimethylnitrosamine-induced Hepatic Fibrosis in Rats.

  Biol. & Pharmaceut. Bull. 28(3), 442-448, 2005.

Commercial Application of Research Projects

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1. Hepatoprotection

Kinsenoside which is a major component of *A. formosanus* shows a novel antifibrotic agent for prevention and treatment of liver fibrosis

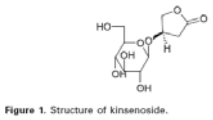
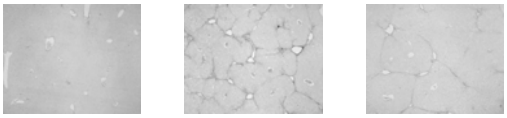


Figure 1. Structure of kinsenoside.

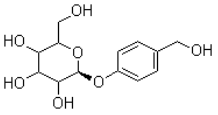


Control CCl4 + H2O CCl4 + AF 1 g/kg

Liver fibrosis

2. Antiamnesic effect

Gatrodin which is a component of *A. formosanus* attenutes amnesia induced by scopolamine

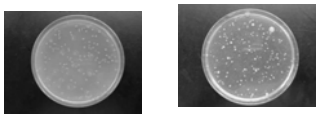


Gastrodin

3. Prebiotic effect

AF stimulates the growth of *bifidobacteria* in rats.

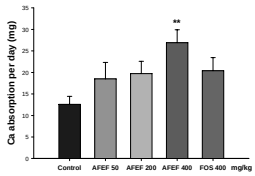
Bifidobacteria

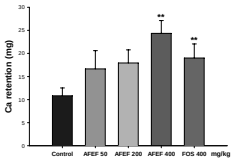


Control AF 0.2 g/kg

4. Bone health

Long term beneficial effects of AF on bone health have been indicated by accumulation of bone mineral content in growing rats or prevention of bone loss in ovariectomized rats

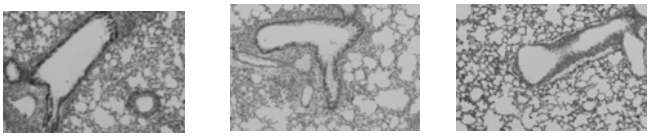




growing rats

5. Immunomodulation

AF attenuates the ovalbumin-induced airway inflammation in mice.



Control OVA + H2O OVA + AF 1 g/kg

airway inflammation


國科會農業生物技術國家型科技計畫
「台灣金線連保肝健康食品之研發」92年度第一次工作進度檢討會議
有容農業生物技術產銷班


有容金線連產銷班第四
049-2986487 0928-366066



Commercial Application of Research Projects

1. *Dendrobium huoshanense*（Yang-Ming University）
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Stage 1 Pioneer Projects

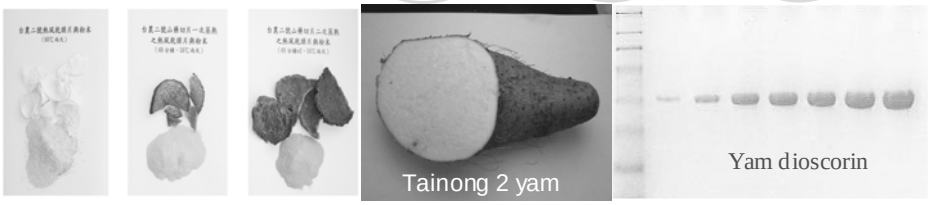
Effects of yam tuber storage proteins, dioscorins, on the antihypertensive activity in spontaneously hypertensive rats (NSC92-2317-B-038-001-) 92/08/01~93/07/31

Publication: Effects of tuber storage protein of yam (*Dioscorea alata* cv. Tainong No.1) and its peptic hydrolysates on spontaneously hypertensive rats. **J. Sci. Food Agric., 2006, 86: 1489-1494.**

Stage 2 Technology Extension and Application Projects

The application of yam in development of healthy food for cardiovascular disease prevention ---Evaluation in blood viscosity improvement and hypertension regulation (95AS-6.2.1-ST-a5) 95/04/01~95/12/31

PI: Hou, Wen-Chi (侯文琪） Liu, Der-Zen（劉得任） supported by AOC/AGV Products Corp. (愛之味食品股份有限公司）



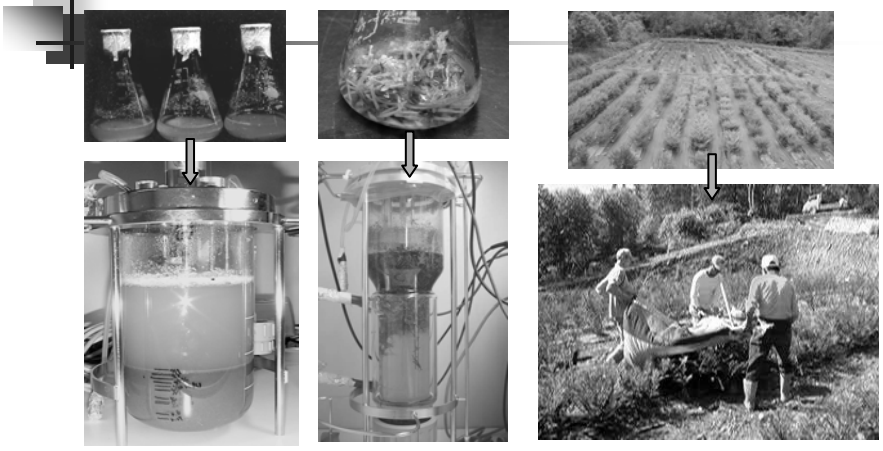
For **antihypertensive effects** of yam processed samples on SHR, it was found that air-dried powders of yam slices (60℃for 2days, 40 and 60 mg/Kg SHR); cooked and then air-dried powders of yam slices (50℃for 2day, 80 mg/Kg SHR) showed the antihypertensive effects during 24-h by oral administrations once. The water extracts heated at 90℃for 10 min was used for long-term oral administrations once a day. It was found that at present data the feeding trial exhibited apparently **lowered blood pressure** than that of the control.

Regarding the **improvement of hemorheological abnormalities**, we used 24-months old aging rats and 8-week diabetic rats as the experimental animal model. The results showed that after the treatment of dioscorin for 21 days the **abnormal hemorheological properties were significant improved** in either aging rats or diabetic rats including a **decrease in blood viscosity and erythrocyte aggregability,** and an increase in erythrocyte deformability and oxygen carrying capability.

Commercial Application of Research Projects

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10. Coconut（Yang-Ming University）

Taxol production models from *Taxus mairei*



From flasks to stirred bioreactor
5~7 mg/L/d baccatin III

From flask to air lift bioreactor
1~2 mg/L/d taxol

From extensive cutivation of superior clones，**2 kg taxol /ha/yr since 3 yr plantation**

Commercial Application of Research Projects

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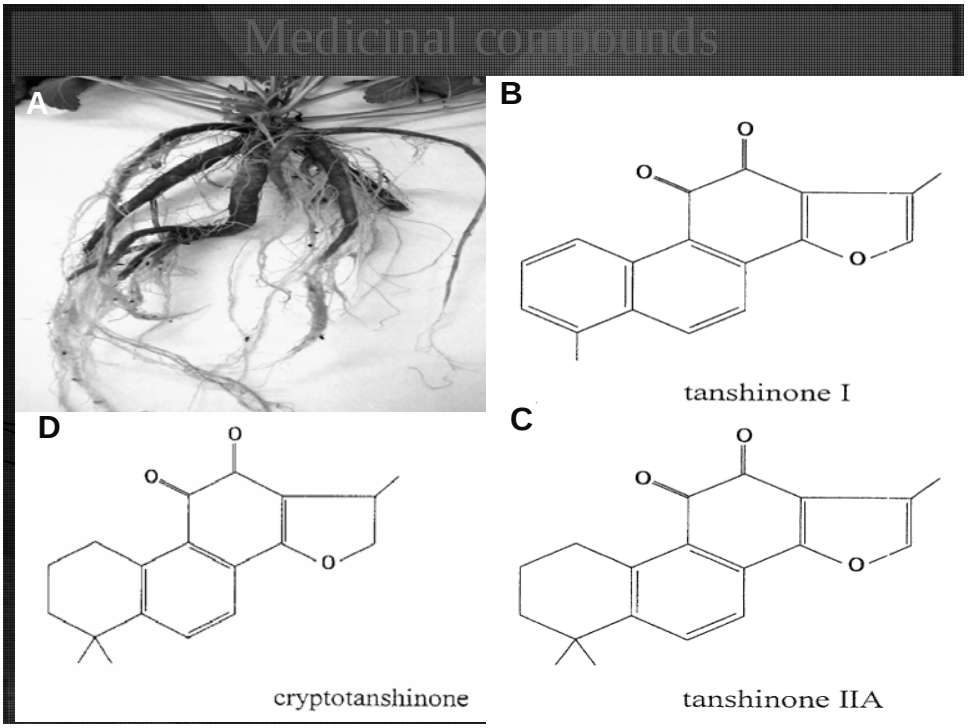
Salvia miltiorrhiza Bunge





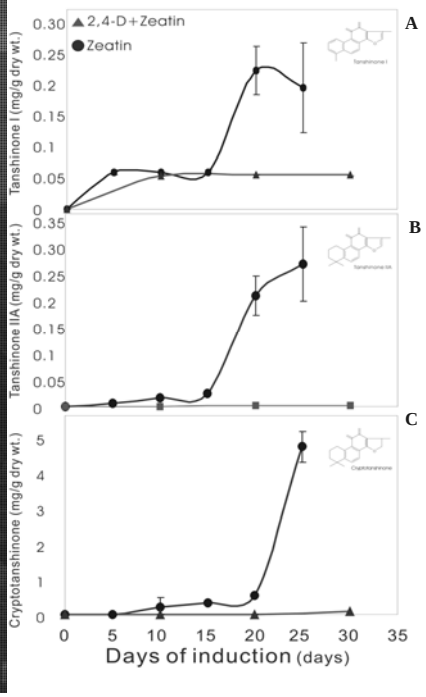
Water-Soluble Extracts of *Salvia miltiorrhiza* Bunge

- Reduced erythrocyte membrane MDA levels
- Increased erythrocyte deformability and decreased erythrocyte flow resistance
- Reduced erythrocyte aggregation、 blood viscosity and blood viscoelasticity
- Increased oxygen-carrying capability of whole blood

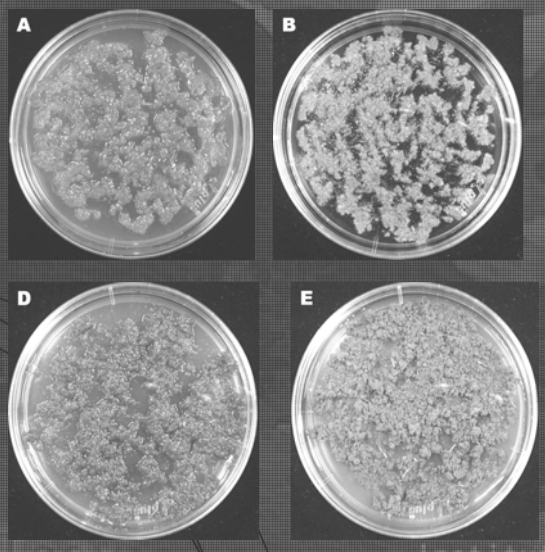


Medicinal compounds induced from callus culture

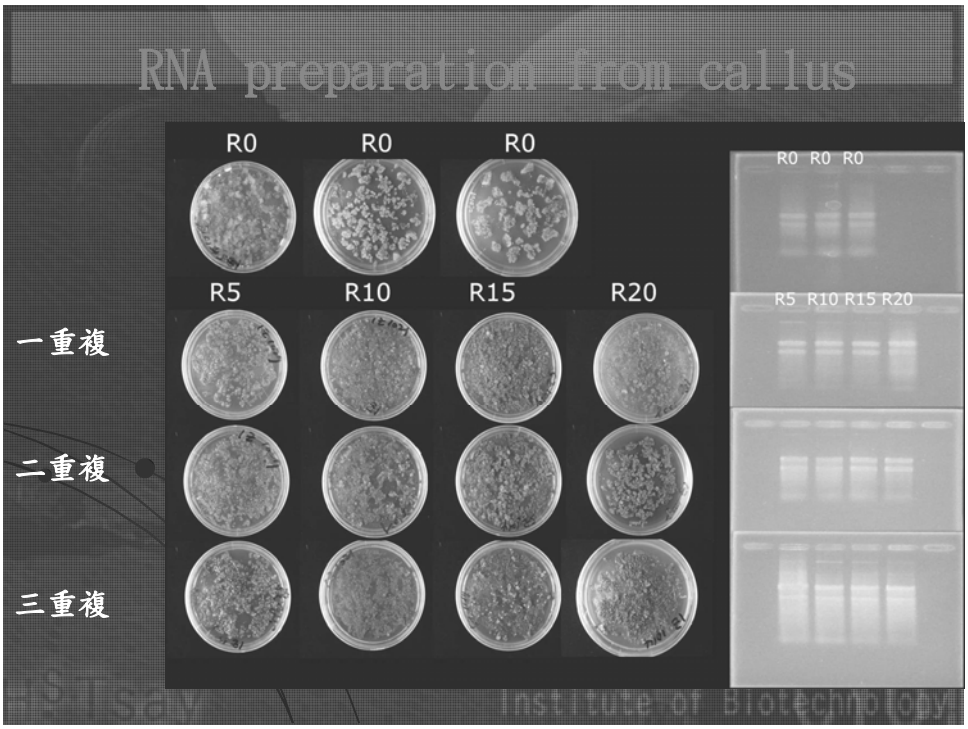
- Zeatin誘導產生隱丹參酮、丹參酮 IIA與丹參酮I之含量變化曲線圖(圖A、B、C)。
- 圖A：丹參癒合組織培養於含Zeatin之培養基經20天後，丹參酮 I之含量可產生0.25 mg/g，丹參癒合組織培養於含2,4-D與Zeatin之培養基30天後，發現隱丹參酮含量僅有0.05 mg/g。
- 圖B：丹參癒合組織於Zeatin處理下丹參酮IIA增加0.3 mg/g。
- 圖C：丹參癒合組織於Zeatin處理下隱丹參酮增加4 mg/g

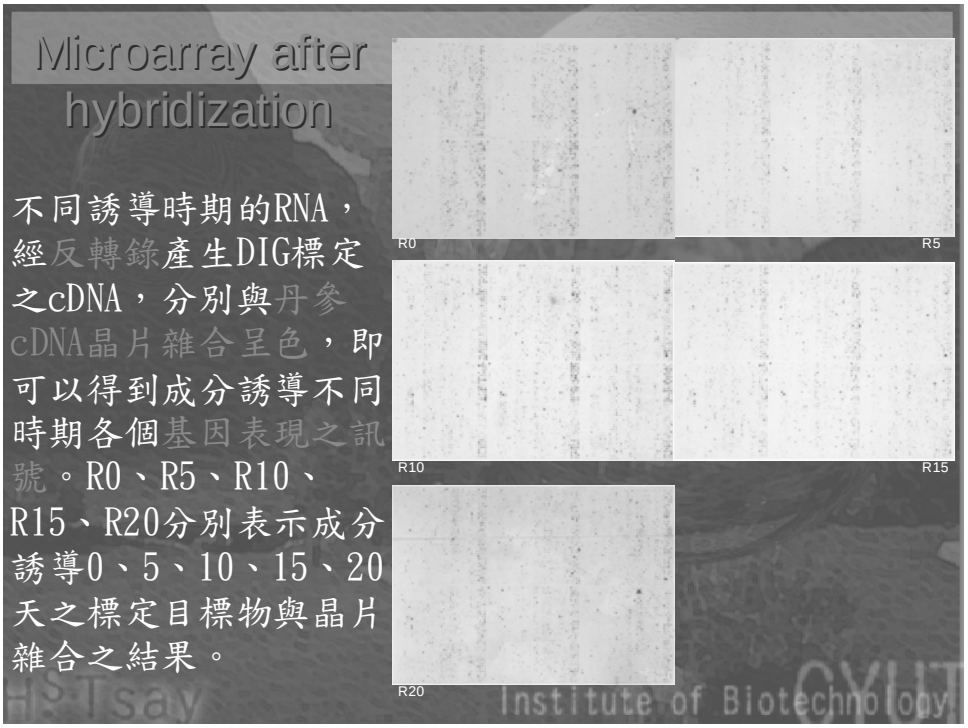


Morphological change from callus cultured in zeatin containing medium



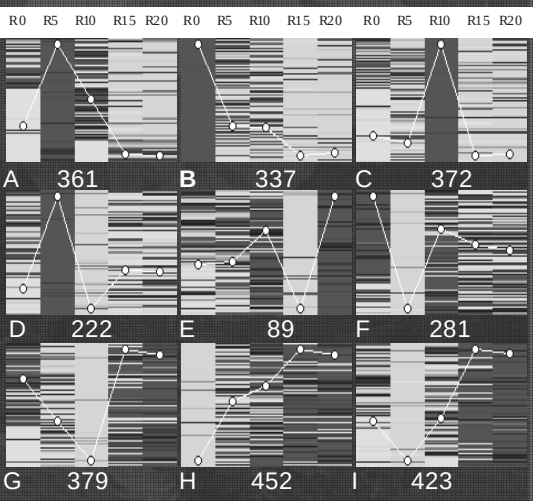
丹參癒合組織經含植物細胞分裂素Zeatin之培養基處理不同天數： A：培養5天、B：培養10天、C：培養15天、D：培養20天、E：培養25天後，細胞外觀色澤變化會因培養之天數增加，而漸漸出現深紅色之癒合組織。


RNA preparation from callus
R0
R0
R0
R5
R10
R15
R20
一重複
二重複
三重複
R0 R0 R0
R5 R10 R15 R20
Institute of Biotechnology


Microarray after hybridization
不同誘導時期的RNA，經反轉錄產生DIG標定之cDNA，分別與丹參cDNA晶片雜合呈色，即可以得到成分誘導不同時期各個基因表現之訊號。R0、R5、R10、R15、R20分別表示成分誘導0、5、10、15、20天之標定目標物與晶片雜合之結果。
R0
R5
R10
R15
R20
Institute of Biotechnology

Analysis after clustering

各時期三重複的雜合晶片經由掃描器掃描成圖片，以GENEPIX軟体讀取圖片產生數據。再利用ACUITY軟体統計分析數據，找出與誘導模式相關連的基因表現輪廓；此圖是以SOM (self-organizing map analysis) 3X3 clusters Euclidean squared統計方法進行分析，分析後可以歸納產生3X3種的基因群表現趨勢(A,B,C,D,E,F,G,H,I等九群)；最上方的橫行R0、R5、R10、R15、R20分別表示不同誘導時期；以A群為例，該群的361個基因，在誘導處理後第5天基因表現達到最高，隨後表現量漸減，第15-20天該群基因表現量會降到最低點。

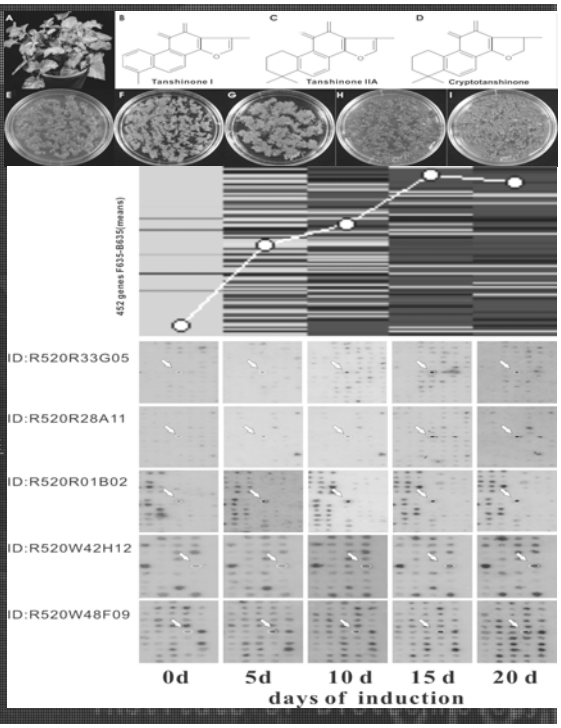


Gene expression

經由丹參成份誘導模式建構之刪減基因庫生物晶片進行雜合分析。SOM 3X3 clusters分析產生9種基因表現群組中，找出與誘導模式可能相關的表現輪廓，挑出訊號數值相差達到二倍以上之基因，並對應雜合相片相符者，A圖即是基因表現在誘導模式中隨著誘導天數的增加，其表現量亦有隨著增加的趨勢；B圖則是從該表現群中選出5個基因

ID:R520R33G05、
ID:R520R28A11、
ID:R520R01B02、
ID:R520W42H12、
ID:R520W48F09，

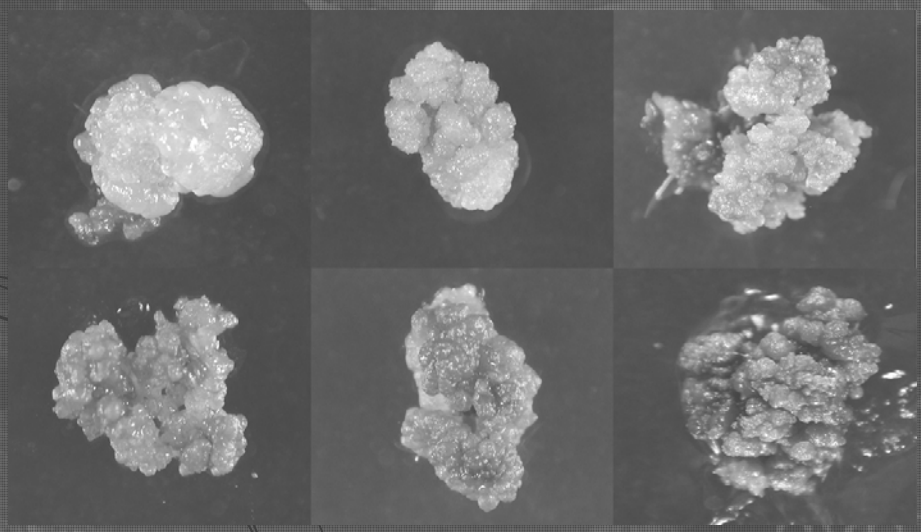
各個誘導時期雜合晶片上的訊號是否與趨勢相符。



基因序列比對

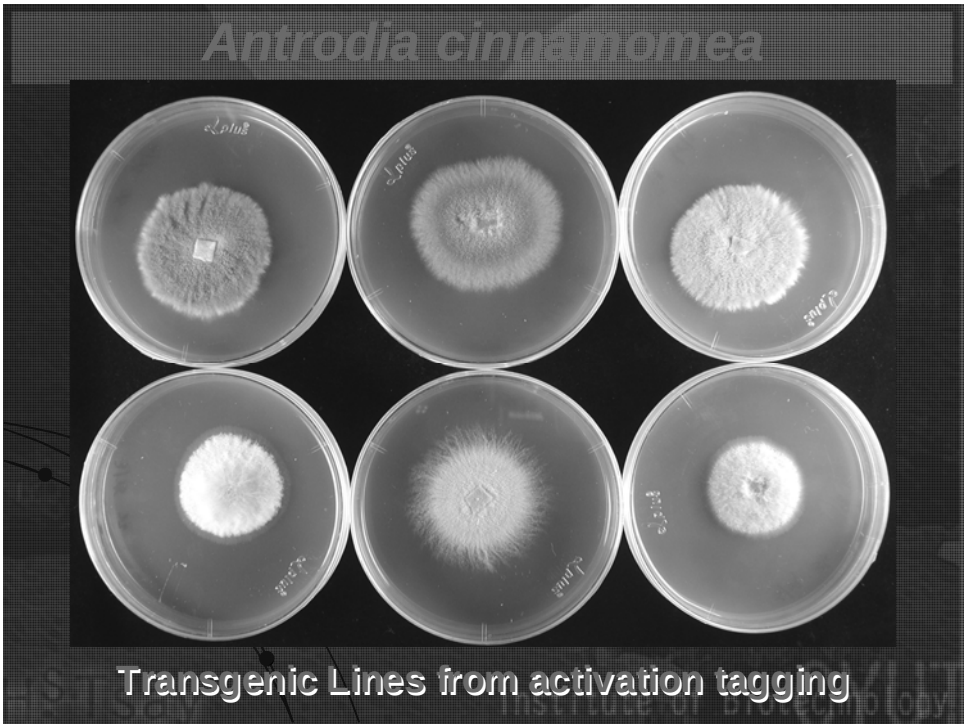
- 利用DNA array分析選出有明顯表現差異62個基因，
- 經序列分析與NCBI基因庫比對結果推測39個不同功能基因。
- 14個基因片段序列其相似性可達75%以上，包含與植物二次謝物相關的monooxygenase, Catharanthus roseus mRNA for cytochrome P450, Catharanthus roseus 10-hydroxygeraniol oxidoreductase, 以及glandular trichome等基因，
- 18個相似性較低的基因片段亦包含與植物二次代謝物生合成相關的基因。
- 功能未知的基因則有4個，其中3個具有75%以上的序列相似性。
- 分析62個基因序列含有23個片段為重複序列，推算cDNA基因庫約含有40%複基因。

Transgenic lines from activation tagging

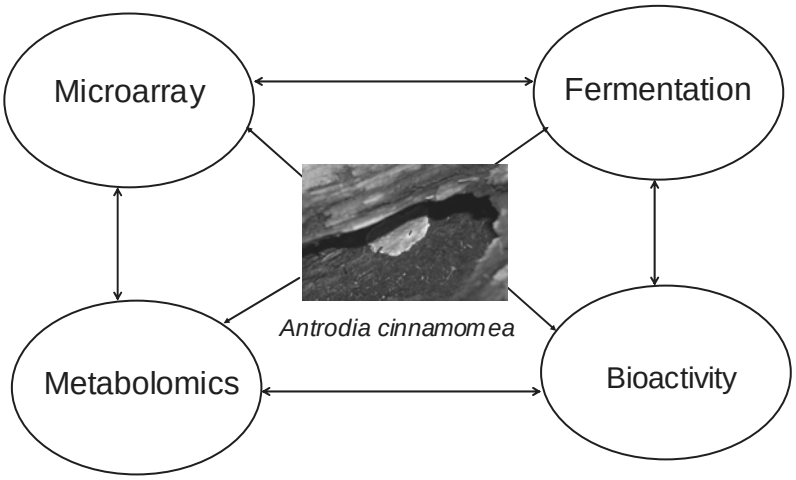


Commercial Application of Research Projects

1. *Dendrobium huoshanense*（Yang-Ming University）
2. *Bupleurum Kaoi*（Kao-Hsiung Medical University and Taiwan Agricultural Research Institute ）
3. *Cordyceps sinensis*（Taiwan Sugar Company）
4. *Anoectochilus formosanus*（China Medical University）
5. Yam for health cooky（Taiwan Agricultural Research Institute）
6. Yam for cardiovascular diseases（Taipei Medical University）
7. Yam (Selection of multiple genotypes and GAP)（Taiwan Agricultural Research Institute）
8. *Taxus mairei*（Taiwan Forestry Research Institute）
9. *Antrodia cinnamomea*（Chung-Hsing University）
10. Coconut（Yang-Ming University）



Establishment of Industrial System for Healthy Food, *Antrodia cinnamomea*





Differential expressed genes

Homocysteine methyltransferase

Polyphenol oxidase

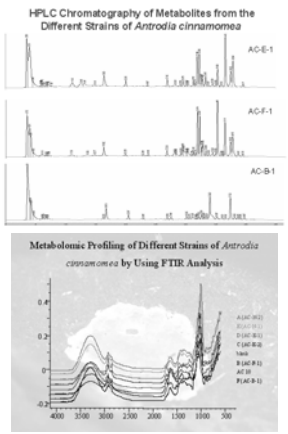
Proteasome maturation factor ump1

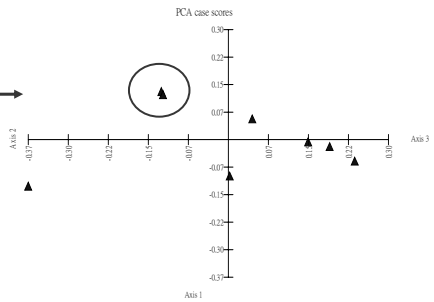
Oligopeptide transporter 7

Nucleoporin p58/p45

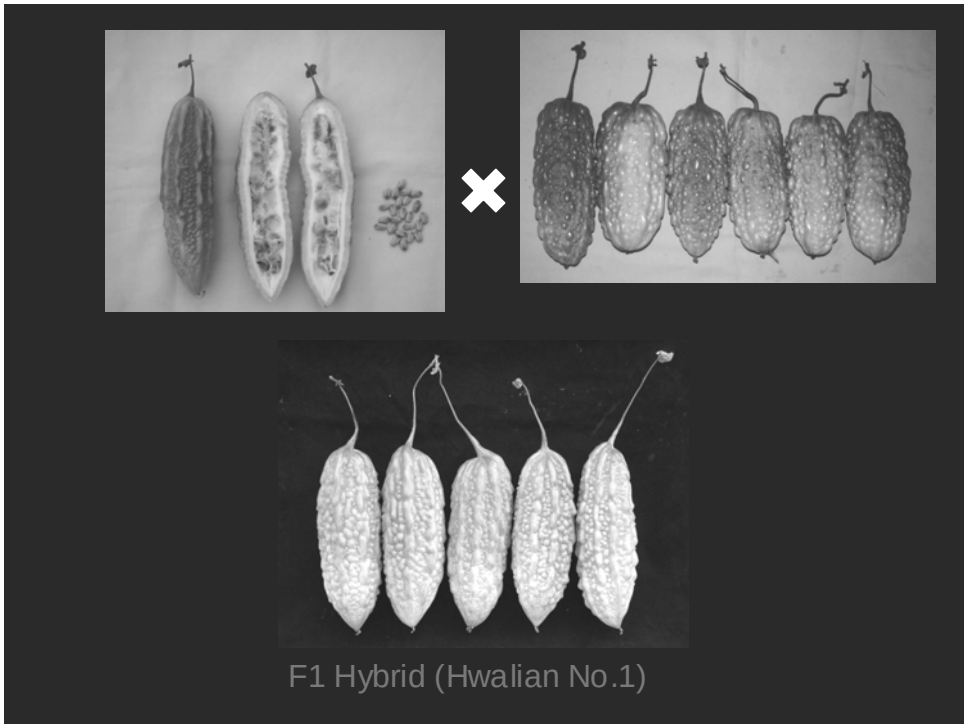
6 Unknown genes

18 Novel genes






Bitter Gourd
10cm
Commercial variety
2cm
Wild spp.


F1 Hybrid (Hwalian No.1)



Field growth of Hwalian No.1



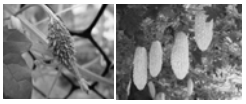
Field growth of Hwalian No.2



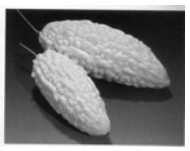
Bitter Gourd

(Bitter Melon, Karela, etc.)

Momordica Charantia



1. Extract of Bitter gourd activates **PPARα and PPARγ**, nuclear receptors that regulate homeostasis of lipid and glucose metabolism.
2. **Identification of 9c, 11t, 13t-conjugated linolenic acid (CLN) as one of the active compounds. Searching of more active compounds is still ongoing.**
3. **In high fat diet induced metabolic syndrome in rats and mice, 1% (wt/wt) of lyophilized bitter gourd in the diet effectively ameliorated**
   - ✓**abdominal obesity**
   - ✓ **hyperglycemia**
   - ✓ **hyperinsulinemia**
   - ✓**hypertriglyceridemia**
4. Breeding of wild bitter gourd with high PPAR activity and high yield.





Commercial Application of Research Projects

1. *Dendrobium huoshanense*（Yang-Ming University）
2. *Bupleurum Kaoi*（Kao-Hsiung Medical University and Taiwan Agricultural Research Institute ）
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9. *Antrodia cinnamomea*（Chung-Hsing University）
10. Coconut（Yang-Ming University）



工業技術研究院
Industrial Technology Research Institute

天然物在高值化產業的應用與發展

潘一紅 博士

生技與醫藥研究所
工 業 技 術 研 究 院

97/06/12

花蓮農改場



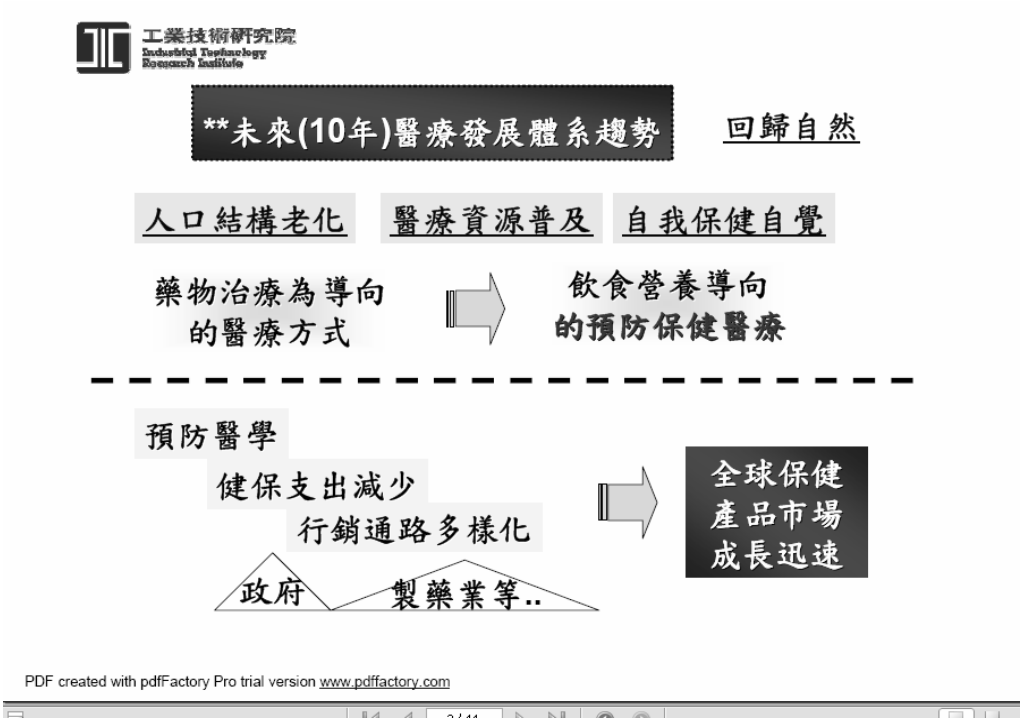
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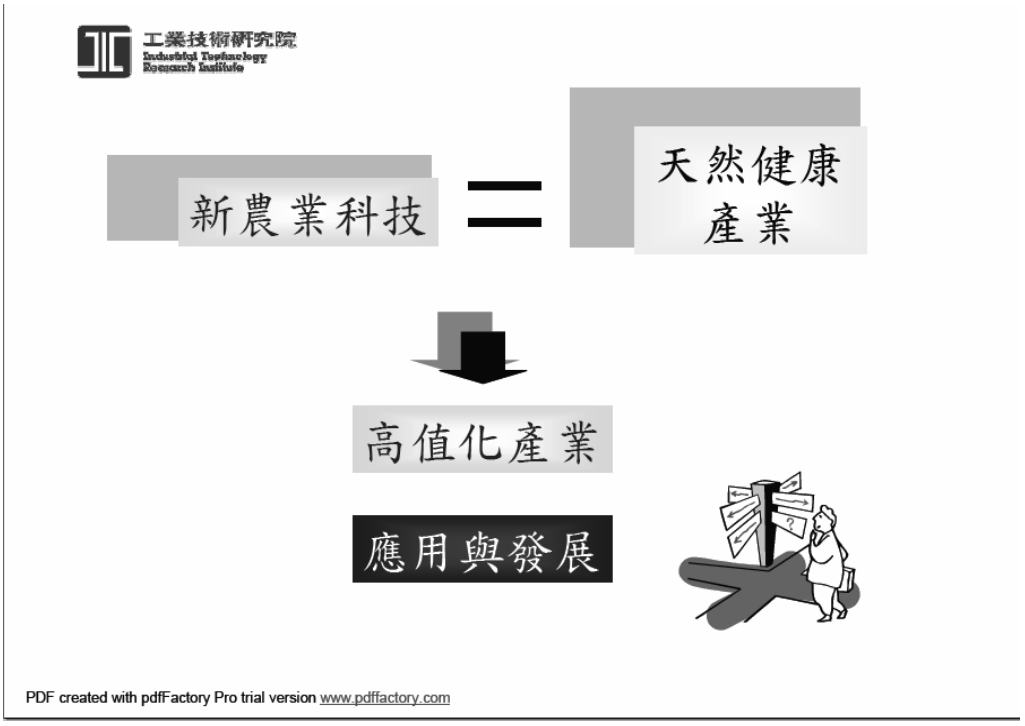
工業技術研究院
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講授大綱

1.前言
2.天然物產業的應用與開發
-健康食品
-天然化妝保養品
-植物新藥
3.預約下一個明星產業

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**未來(10年)醫療發展體系趨勢
回歸自然
人口結構老化
醫療資源普及
自我保健自覺
藥物治療為導向的醫療方式
飲食營養導向的預防保健醫療
預防醫學
健保支出減少
行銷通路多樣化
政府
製藥業等..
全球保健產品市場成長迅速
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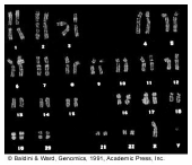

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Industrial Technology Research Institute
新農業科技
=
天然健康產業
高值化產業
應用與發展
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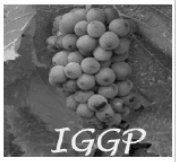
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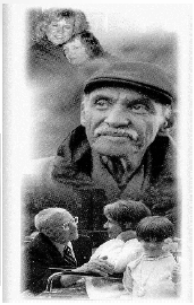
台灣新農業發展

成長理由

- 注重養生，預防，保健觀念增加
- 台灣擁有優異之農業技術
- 老化社會來臨，生技保健產品具相當潛力
- 消費者知識提高，具獨特之農業生技產品需求增加
- 政府對農業生技產業持續支持







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人氣最旺『三隻小豬』

以蘭嶼豬為基礎種畜

迷彩豬

蘭嶼豬　　迷彩豬　　花斑豬



醫學研究用:例如心血管疾病病理機制探討、基因篩選技術研發

細胞與幹細胞移植治療、藥物研發、外科材研開發等等

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工業技術研究院
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農業生技研發實力



人工繁殖『海馬寶寶』



『紅色、綠色螢光寶寶』

⇨ 生產高價值免疫蛋白



『基因轉殖技術』

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研究結果受肯定 登上Nature Medicine期刊

北醫生薑、咖哩萃取物 讓肌肉萎縮症鼠「康復」

台北醫學大學研究團隊日前宣布將一種植物萃取物ASC-J9，注射到肌肉萎縮症的老鼠體內，使原本般體不靈活的老鼠，逐步恢復原有的活動能力！而該項研究成果被刊登在知名期刊Nature Medicine上。

- 脊隨性肌肉萎縮症(SBMA)男性荷爾蒙受體的胺基酸多達40幾個,太長的a.a.捲成一團，並集中在神經元細胞上，使神經無法正常運作
- ASC-J9可分解男性荷爾蒙受體的胺基酸，為治療SBMA帶來希望
- 保留患者性功能避免『去勢』



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工業技術研究院
Industrial Technology Research Institute

天然物產業的應用與開發

-健康食品

-天然化妝保養品

-植物新藥



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工業技術研究院
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高值化食品

分類

健康食品
- 經功效安全驗證
- 可訴求療效

機能性食品
- 含機能性成分
- 不可訴求療效

健康概念食品
- 一般優質食品
- 有機/新鮮/強化營養

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•2006年全球保健市場約2200億美元

•預估2015年將超過一兆

表一：美國營養補充品銷售前十五項產品機能及比率

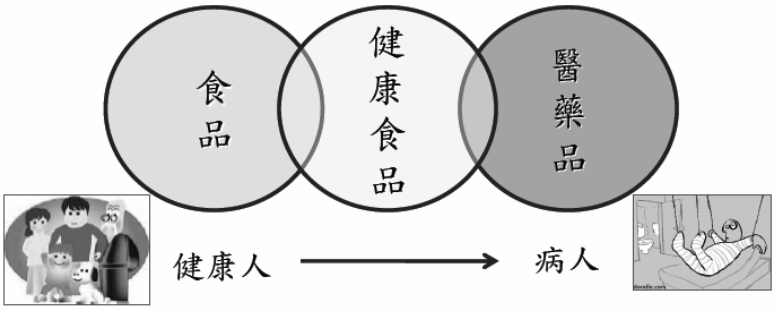
排名序	產品機能	所佔比率(%)
1	運動、減肥相關	37
2	滋養強壯	25
3	關節健康相關	7
4	免疫、感冒	5
4	心血管相關	5
4	骨骼相關	5
5	抗癌	3
5	糖尿病	3
6	整腸	2
6	肝臟	2
6	智力	2
6	更年期障礙	2
7	失眠	1
7	眼睛保健	1
7	性功能提昇	1

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預防勝於治療-健康食品

不可宣稱有療效，更不可替代正規的醫療



(1)安全的 (2)營養的 (3)能預防疾病的

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◎健康食品定義：

提供特殊營養素或具有特定保健功效，非以治療為目的的食品

◎健康食品管理法-88年8月3日實施（健康食品=法定名詞）

◎由衛生署食品處嚴格審核把關

◎推動健康食品雙軌制



健康食品之標準圖樣



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健康食品管理現況

1.腸道健康	2.調節血脂
3.免疫調節	4.護肝功能
5.骨質保健	6.牙齒保健
7.調降血糖	8.抗疲勞
9.延緩衰老	10.輔助調節血壓
11.促進鐵吸收	12.輔助調整過敏體質
13.不易形成體脂肪	

116件健康食品許可證
(97/04/02)

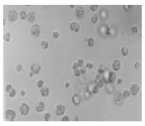


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GAP → GMP → GLP → 認證

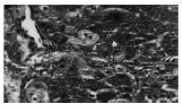
基源鑑定 製造品管 功效安全 上市



Identify test



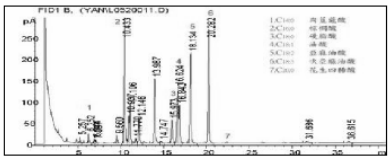
GMP process



Efficacy test

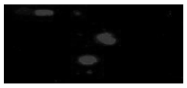


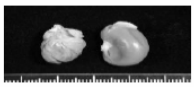
Certificate



Quality control







Toxicity test

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代謝症候群(Metabolic Syndrome)

- 肥胖
- 高血脂
- 高血糖
- 高血壓



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我國血脂異常分類及建議之藥物治療

	膽酸結合樹脂類廣泛使用之藥物 (bile acid sequestrant resin)	纖維鹽酸衍生物類 (fibric acid derivatives)	菸鹼酸類 (nicotinic acid)	HMG-CoA reductase statin)	Omega-3 fatty acid
高膽固醇血症 (總膽固醇TC ≥200mg/dl)	V	V	V	V	
混合型高血脂症 (TC ≥200mg/dl，TG≥ 200mg/dl)		V	V	V	
高三酸甘油酯血症 TG ≥ 200mg/dl，且合併 TC/HDL ≥5或 HDL＜ 40mg/dl		V	V		V

＊資料來源：行政院衛生署國民健康局

＊與歐洲動脈硬化學會分類同

*高血脂症(hyperlipidemia)治療，第一優先考慮飲食控制、減重等，治療3-6個月後，再視情況採用藥物治療。

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天然物與降血脂研發

- 機能性成分/素材

大豆蛋白(soy protein)、褐藻酸鈉(sodium alginate)、甲殼素(chitosan)、車錢子種皮纖維(psylium)、銀杏葉、綠茶、芸香苷、EPA、DHA、納豆激酶、輔酵素Q10、低聚糖、桑葉、紅蚯蚓、蕎麥、盧丁、亞麻油、植物固醇(plant sterols)、大蒜、紅麴

	保健功效	活性成分物質
桑葉	降血糖	生物鹼、桑葉多糖
	降血脂	植物甾醇
	降血壓	γ-氨基丁酸
	老化	異黃酮

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糖尿病的診斷標準

根據統計，全球罹患糖尿病的人數高達1.35億~1.6億

1.任何時間血糖值超過200mg/dl且有糖尿病典型症狀
"多吃、多尿、多喝、體重下降等症狀。
2.空腹8小時以上之血糖值≧126mg/dl
3.口服葡萄糖耐量試驗(OGTT)後2hr血糖值≧200mg/dl
(服用75g)
正常人空腹血糖值約90mg/dl，飯後血糖＜140mg/dl

根據1997美國糖尿病學會訂定標準，若符合上述
任何一個條件，經再次確認即可診斷為糖尿病

◆最可怕的是會引起一些 併發症

◆以飲食療法為最基本也是最重要的療法

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Industrial Technology Research Institute

天然物與降血糖研發

- 機能性成分/素材

苦瓜(*Mormordica charantia*)、武靴葉(*Gymnema sylvestre*)、葫蘆巴(*Trigonella foenum-graecum*)、蘆薈(*Aloe vera*)、大蒜(*Allium*)、鉻（chromium）等。









Diachrome® Diabetes Essentials™
Blood Sugar Health

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合作案例-植物性健康食品開發與認證

國內首例藻類
健康食品認證通過



國內首例中草藥
複方健康食品



護肝類健康食品





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50年內 英國將有250萬人罹患癡呆症

肥胖可能導致癡呆症？

科學家最近推測表示，隨著肥胖比率的提高，人們罹患阿滋海默氏症（Alzheimer's disease）的數目會越來越高。據BBC報導，英國「阿滋海莫氏症協會」（Alzheimer's Society）提出警告，若再不徹底解決肥胖問題，以英國為例，50年內將有高達250萬人可能罹患癡呆症。

增加飽食感:如西洋車前子、聚葡萄甘露糖等。
抑制醣類吸收:武靴葉、桑葉等。
促進醣類代謝:羅望子果、桂皮、啤酒酵母等。
抑制脂肪吸收:如藤黃果、葛根、左旋-肉鹼等。
促進脂肪燃燒:如膽鹼、金桔、辣椒等。



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天然物產業的應用與開發

-健康食品

-天然化妝保養品

-植物新藥



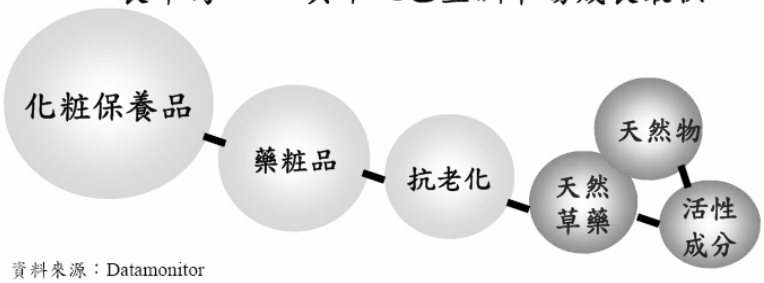
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工業技術研究院
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全球化粧保養品市場規模

2008年預估1,780億美元，亞洲部分，2008年預估可達490億美元，約佔全球市場的27.8%。

•Goldman Sachs估計全球化粧品業年平均成長率為7%，其中又已亞洲市場成長最快。



資料來源：Datamonitor

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工業技術研究院
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生技美容科技發展與趨勢

配合化學、化工、生物技術、生物材料、幹細胞及皮膚醫學等發展使醫學美容更有深度

生物醫學美容 ➡

- 重組基因工程及細胞工程發展
- 基因工程產品研發應用:胰島素、生長激素、干擾素、EGF、FGF、TGF等生長因子
- 如利用基因生長因子 和基因工程重整骨形成蛋白(BMP)進行組織修復/美容修復

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生技美容科技發展與趨勢

幹細胞美容 ➡
- 胚胎幹細胞襲捲全球
- 醫學美容

基因治療美容 ➡
- 基因時代/基因治療替代
- 引起美容缺陷基因調控

天然草本美容 ➡





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工業技術研究院
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皮膚保養品的負擔

- 介面活性劑/香精/防腐劑
- 容易過敏物質：花生油/綿羊油

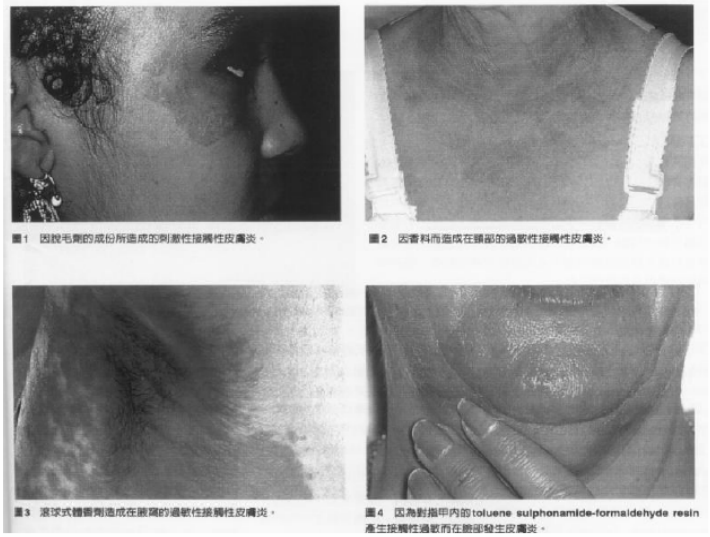


圖1 因脫毛劑的成份所造成的刺激性接觸性皮膚炎。
圖2 因香料而造成在腰部的過敏性接觸性皮膚炎。
圖3 滾球式體香劑造成在腋窩的過敏性接觸性皮膚炎。
圖4 因為對指甲內的toluene sulphonamide-formaldehyde resin產生接觸性過敏而在腋窩發生皮膚炎。

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天然化粧品

綠色化粧品旗艦品牌－THE BODY SHOP



明亮簡單的店面

at home 的銷售手法



植物配方的產品系列



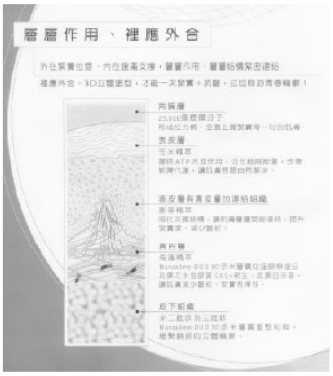
資料來源：THE BODY SHOP International

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工業技術研究院
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時尚化妝品流行之天然成分





玉米/海藻/麥芽/Peptide

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天然來源化妝品成分

緩和日曬/抗發炎作用

*洋甘菊萃取物類(Chamomile ET)

係自菊科植物甘菊花之乾燥花抽提出來之成分，具有阻止皮膚黑色素之生成作用，減少紫外線照射後表皮細胞所致之色素沉著，可預防因太陽照射而產生黑斑、雀斑。
衛生署公告《 中華民國八十九年六月》
*Chamomile ET 0.50%



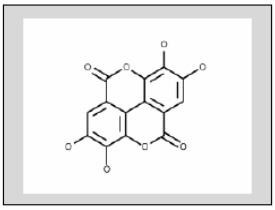
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工業技術研究院
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天然來源化妝品成分

*鞣花酸(Ellagic acid) 美白/抗發炎作用

- Commonly occurring plant phenol
- raspberry(覆盆子)、草莓及櫻桃
- 衛生署新公告美白成分(2004/10/19)
- 使用限量0.5%
- 在日本已使用多年（LION專利）
- 高絲向衛生署提出申請





4,4',5,5',6,6'-Hexahydroxydiphenic acid 2,6,2',6'-dilactone

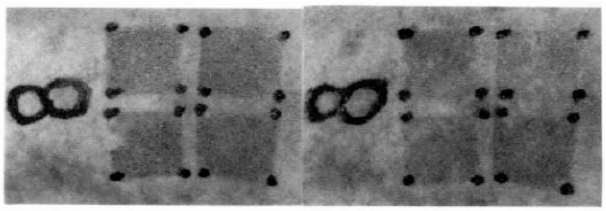
$C_{14}H_6O_8$, M.W.302.19

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工業技術研究院
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*鞣花酸(Ellagic acid)

Prevention of skin pigmentation effect induced by UV-light irradiation



4 weeks 6 weeks

- UV-B irradiation(8 days)
- Guinea-pigs
- 動物試驗具美白效果

1% EA

Vehical	Negative control

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*鞣花酸(Ellagic acid)

Tyrosinase activity assay in skin tissue
-Dopa Reaction

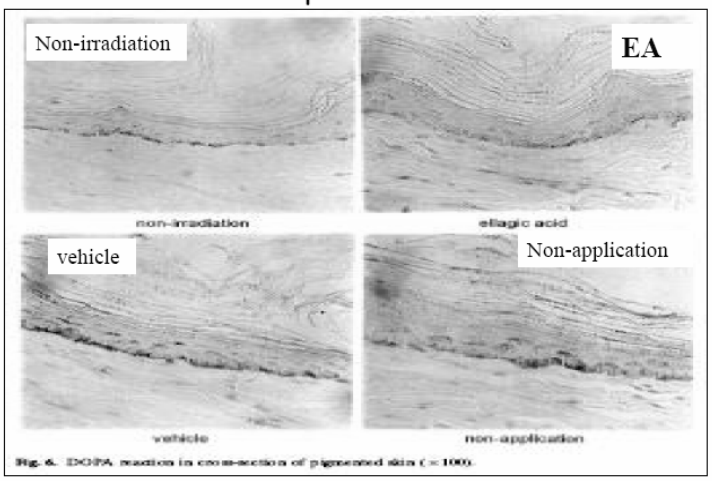


Fig. 6. DOPA reaction in cross-section of pigmented skin (×100).

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*鞣花酸(Ellagic acid)

Comparison of lightening effect of some
commercially available susbtance

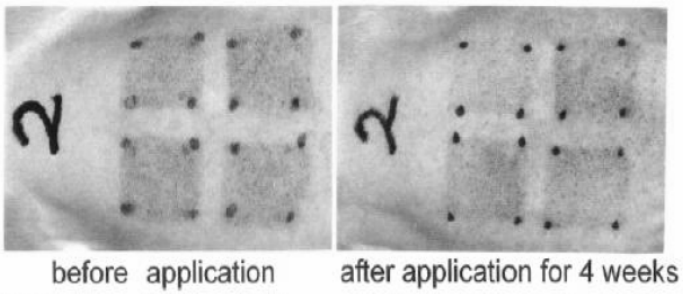


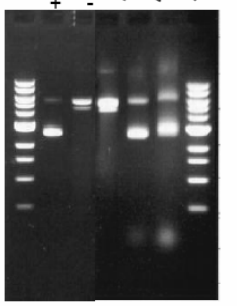
Fig. 9. Comparison of lightening effect of some commercially available agents in skin pigmentation induced by UV-light irradiation. Samples were applied for 4 weeks after UV-light irradiation (eight times). Upper left; ellagic acid (1%), upper right; vehicle, lower left; arbutin (1%), lower right; kojic acid (1%).

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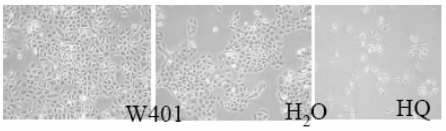
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合作案例-植物性化粧品天然原料及產品開發

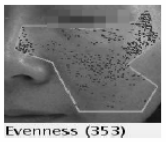
基因防護試驗

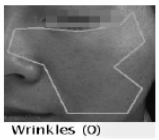


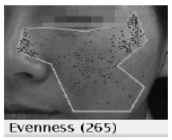
角質細胞











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天然物產業的應用與開發

-健康食品

-天然化妝保養品

-植物新藥



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植物藥發展之主要因素

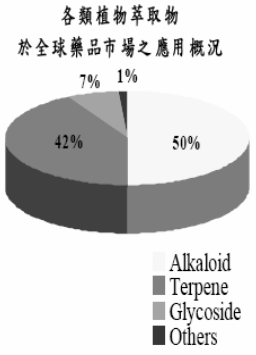
- ✓ 西藥常有副作用，以傳統療法做輔助治療增加醫治機會。
- ✓ 從天然物中尋找可能治療之高傳染性疾病(如SARS、AIDS等)或慢性疾病成分，不失為一種新的研究方向。
- ✓ FDA已提出並公布 **"Guidelines of the Botanical Drug Industry"**，國內衛生署亦已公告「中藥新藥查驗登記須知」、「中藥藥品臨床實驗申請作業相關規定」等。
- ✓ 西藥化學合成小分子的新藥開發，不但耗時且需投入大量資 金，開發速度緩慢。
- ✓ 新藥篩選的方法日益進步，靈敏且速度快(如high throughput screening)。

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植物藥市場現況

*全球約有250,000類植物，已開發之植物約有200種。



資料來源：BBC

**2006/323億美元
2007/362億美元
**2003~2007年平均成長率12%
**美國佔有率達29%，其次為德、法及亞 洲的中國大陸及日本

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植物新藥及西藥研發時程比較

- 西藥新藥開發流程：研發時間9 -15年，金額3.6-5億美元
- 植物新藥開發流程：研發時間4 - 7 年，金額300萬美元

新藥探索階段 → 臨床前試驗藥理試驗 → 試驗中新藥IND * → 臨床試驗Phase Ⅰ → 臨床試驗Phase Ⅱ → 臨床試驗Phase Ⅲ → 新藥申請NDA * *

投入經費（百萬美元）	西藥	25-30	30-35	80-90	200	5-10
	植物新藥	0.1-1.0	-	1.5-5.0	15.0-20.0	1.0-1.5
時間（年）	西藥	5-7	0.6-1	1-2	2-3	1-2
	植物新藥	0.5-1.0	-	1.0-3.0	2-3	0.5-1.0

＊ IND (Investigational New Drug)：申請進行人體臨床試驗
＊ ＊ NDA (New Drug Application)：申請新藥上市與新藥審查
資料來源：生技中心 IT IS 計畫整理

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基改作物大翻身！

利用植物製造的胰島素即將問世？

英國媒體BBC報導，加拿大生技公司Sembiosys可能於3年內，在市面上推出由基改植物所製造的胰島素（insulin）。

- 人工胰島素大多經由基改細菌於密閉槽中製造。加拿大生技公司Sembiosys研究在開放空間中由基改植物製造胰島素(insulin)。
- 基改紅花(safflower)種子中萃取insulin，目前已在智利、美國及加拿大實驗田展開種植。



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工研院與植物新藥開發



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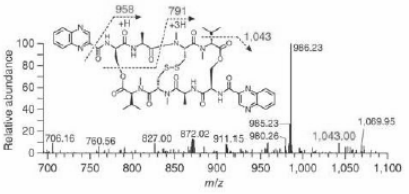
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植物新藥開發

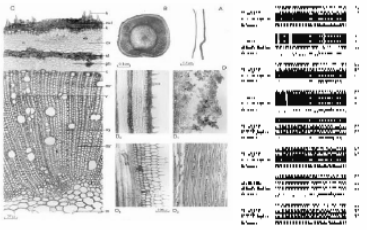
天然物原料、製程中管制、活性指標成份分析鑑定



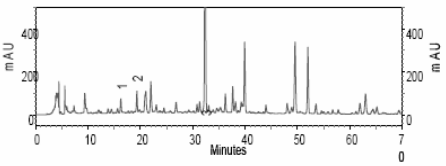
LC/MS
GC/MS
NMR



結構鑑定



組織切片、基源鑑定



指紋圖譜

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植物藥發展趨勢

- ✓ 單一成分植物藥開發：傳統的複方草藥製劑由於成分複雜，難以科學化管理，具醫療活性的成分篩選較熱門。
- ✓ 各大藥廠爭相投入研發西藥尚無法治癒之疾病，如癌症、AIDS、SARS、肝炎等領域。
- ✓ 慢性病，如糖尿病、心血管疾病、怕金森氏症等慢性疾病，是現代文明病。植物新藥的使用也許可達到保健及疾病根治的目標。
- ✓ 法規的建立：全球各國對於植物藥的分類及管理不盡相同，造成這類藥品在應用上受限。
- ✓ 科學化驗證：依據西藥臨床試驗之精神管理植物藥，使植物新藥的使用更為安全、有效。

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預約下一個明星產業 -展望與機會



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苦瓜新品種育成及 GAP 體系之建立

全中和、林砡竹

行政院農業委員會花蓮區農業改良場
作物改良課 副研究員及專任助理

摘　要

花蓮區農業改良場選擇優良的地方種山苦瓜及野生苦瓜利用雜交育種技術於 2006 年-2008 年間育成「花蓮 1 號」、「花蓮 2 號」、「花蓮 3 號」及「花蓮 4 號」之山苦瓜品種，新品種採 GAP 生產提供作爲生物活性及相關試驗用。GAP 體系之建立從 2006 年開始選擇苦瓜新品種「花蓮 1 號」及「花蓮 2 號」於新竹、雲林、花蓮及高雄進行一年兩個期作之試驗；2007-2008 年選擇苦瓜新品種「花蓮 2 號」「花蓮 4 號」於苗栗、花蓮進行二年各兩個期作，於高雄進行二年各四個期作之試驗，選定農民及其農地，以有機栽培方式進行生產，依規定經採取土壤、肥料、水質檢測皆符合標準，方進行種植，試驗探討不同苦瓜品種於不同地點、期作、採收期及連作與輪作之耕作制度及低度肥培之栽培管理生產下，其穩定性與活性。同一品種經過 2 年在北部、中部、東部及南部種植，其果型、果色、果實大小皆無變異，顯示苦瓜新品種之基質穩定。不同苦瓜品種於不同地點、期作、採收期有不同活性。連作與輪作栽培管理對植株生長影響不大。產量以高雄試區表現最好，最高公頃產量超過 61 公噸。就其生長勢及產量之表現及氣象溫度變化知北部、東部最適當的栽培期間 3-10 月；南部全年皆可種植。

關鍵詞：苦瓜、優良種植規範

前言

苦瓜（Bitter gourd）又名錦荔枝、癩葡萄、癩瓜等,學名爲 *Momordica charantia* L.，屬葫蘆科，一年生蔓性草本作物，原產於亞洲熱帶地區，主要栽培於亞洲，爲東方蔬菜之一。台灣地區年栽培面積約 2,000 公頃，營養價值高，爲重要瓜類蔬菜。苦瓜種類繁多，依果色可分爲白色、白綠色、綠色及濃綠色等；就果面突起可分爲珍珠突起、尖銳突起及條肋狀突起；而果實形狀又可分爲紡錘形、短橢圓形、長橢圓形、圓形、三角形等。早期台灣苦瓜以白皮、肉厚、苦味淡較受一般人歡迎，近年來由於多元化食用方式盛行，綠皮尖銳突起之小果型的山苦瓜也漸漸引起消費者的興趣；由於苦瓜爲異交作物，品種間容易產生雜交，不易維持原品種之優良特性，加上目前大部分山苦瓜品種爲農民自行留種，品種容易退化。而山苦瓜具有早熟、多雌花、生長期長等優良特性。因此利用山苦瓜的優良特性加以固定之後，育成優良雜交一代品種，供推廣用，實有其必要性。

現代中草藥之道地藥材除了野生種外，大部分則需要 GAP 的基地來長期培植，GAP（Good Agricultural Practice）爲國際要求的優良種植規範，主要目的是要生產優質、道地、無污染的中藥材，主要分三個體系:藥材品質、操作規範及後期加工標準。從種植、土壤開始，到施肥、農藥、栽培、採收、運輸、儲藏等各個環節都需要有標準作業規範。李並指出道地藥材生長在一個特定的地域，離開了這個特定的地域，改變了生長環境，其有效成份含量就會降低，臨床使用上甚至無效（李 2004）。佘（2004）指出中草藥產業化所面臨的瓶頸，包括基源混亂、品質成分不穩定，沒有自有原料體系。賴（2007）指出栽培技術直接影響到中草藥的產量與品質。以人工栽培方式大量生產中草藥，必須篩選原藥材之優良基原植物，配合精準農業及永續農業的精神。由此可見藥用作物產業 GAP 體系建立之重要及其迫切性。行政院農業委員會花蓮區農業改良場長期進行原生蔬菜育種及栽培技術改進，育成許多山苦瓜固定品系及品種。鑑於未來在進行功能性作物品種推廣時，有必要針對相關作物在個體的作用情況進行科學認知，確定有效成分的作用機制，開發農作物多元化的應用潛力，以確立其有效功能及作用的機制，提供產銷及推廣之參考。苦瓜栽培期間常會遇到蚜蟲、瓜螟、薊馬、粉蝨及瓜實蠅等害蟲爲害與炭疽病、白粉病、露菌病等病害，一般都會施用殺菌劑及殺蟲劑以防止病蟲害，取用這些材料生產保健食品，安全堪慮；因此需建立一套苦瓜 GAP 保健用苦瓜有機量產模式。

苦瓜新品種育成

苦瓜種原的蒐集以花蓮地區農民自行留種栽培的地方種山苦瓜與全省各地野生的小苦瓜爲主，種子蒐集登記編號後種植於本場，選拔生長勢較強，結果數較多，果型較佳的植株給予自交留種，選拔之優良單株，進行 4-5 個期作的自交純化選拔，每期作每個入選株自交留種，於下一期作再種植 10 株，從其中再選拔優良單株自交留種。經過 5 代自交純化之後選擇其中高雌花率、果型整齊的自交品系，進行雜交，F1 組合力試驗、品系比較試驗、區域試驗、栽培密度試驗、截切力測定、最後選出具有生長勢強、雌花早開、高雌花性、結果力強、截切值低、品質佳、口感接近野生小苦瓜等諸多優良特性之苦瓜品種，於 2005 年-2007 年育成「花蓮 1 號」、「花蓮 2 號」、「花蓮 3 號」及「花蓮 4 號」等 4 個山苦瓜品種。經過營養成份分析，「花蓮 1 號」、「花蓮 2 號」、「花蓮 3 號」果實所含之維他命 C、葉酸及膳食纖維皆高出市售白色大苦瓜甚多（表一）。希望在多元化食用方式盛行的今日能讓消費者有更多選擇。

苦瓜GAP生產體系之建立

苦瓜GAP生產模式之建立先選定苦瓜有機栽培農戶，以花蓮區農業改良場育成之苦瓜新品種爲材料第一年探討栽培地點、品種及採收期與生物活性之關係；第二年探討耕作制度及全年種植與生物活性；第三年探討經前2年試驗最具活性及功效之花蓮2號及花蓮4號山苦瓜作爲種植材料，以設施有機栽培方式生產配合精準低投入施肥技術管理生產，探討苦瓜作爲保健材料之最佳種植時期及取材時間，並記錄生產管理過程、產量，及期間所需所有費用，並將不同生育期，不同成熟度之果實提供作爲研究生物活性與功能性基因開發的材料。選定臺灣北部（新竹市（2006）及苗栗縣頭份鎮（2007-2008））、中部（雲林縣西螺鎮（2006））、南部（高雄縣杉林鄉）及東部（花蓮縣壽豐鄉（2006-2007）、玉里鎮（2008））天然環境良好之有機苦瓜栽培農戶，以設施有機方式生產，檢測土壤、施用肥料及灌溉用水之是否含有重金屬，記錄生產管理過程、病蟲害發生及防治，生產量，及其所得，並將不同品種、不同期作，不同生育期、不同成熟度之果實經過農藥殘毒檢測，確定完全無農藥殘留再提供作爲生物活性與功能性基因開發的材料，以確立苦瓜作爲藥用作物或保健作物材料生產之依據。

2006年苦瓜新品種「花蓮一號」及「花蓮二號」經過一年兩個期作於

新竹、雲林、高雄及花蓮進行GAP種植，2007年苦瓜新品種「花蓮二號」經過一年兩個期作於苗栗、花蓮，一年四個期作於高雄進行GAP種植，2008年以苦瓜新品種「花蓮2號」及「花蓮4號」進行GAP種植，依規定選定農民及其農地（表二），以有機栽培方式進行生產，經採取土壤、肥料、水質檢測皆符合標準（表三-五），方進行種植。2006年春作生產以雲林試區表現最好，公頃產量超過35公噸，其次爲新竹試區，公頃產量超過24公噸。夏作生產以高雄及雲林試區表現較好，公頃產量超過10公噸。2007年一期作生產以高雄試區表現最好，公頃產量超過61公噸，其次爲苗栗試區，公頃產量超過58.7公噸。二期作高雄試區連作田植株生育正常，三期作生長採收期遇到科蘿莎颱風爲害，產量皆減少許多（表六）。同一品種經過二個期作在新竹、花蓮二個地點種植，四個期作於高雄試區連作田種植，植株生育正常綜合各期作各試區之園藝性狀調查，其果型、果色、果實大小皆同，顯示苦瓜新品種「花蓮二號」之基質穩定（表七）。

表一、苦瓜營養成份檢測結果比較*

檢驗項目	單位	花蓮一號	花蓮二號	花蓮3號	大苦瓜	野苦瓜
水分	g/100g	90.82	92.14	92.50	-	90.2
灰分	g/100g	0.79	0.60	0.64	0.60	1.21
粗脂肪	g/100g	0.46	0.32	0.24	-	1.2
粗蛋白質	g/100g	1.63	1.51	1.48	-	2.4
粗纖維	g/100g	2.22	1.41	1.96	-	2.5
碳水化合物	g/100g	4.08	4.02	3.18	3.9	-
熱量	Kcal/100g	27	25	21	17	-
膳食纖維	g/100g	4.10	2.72	3.28	0.5	5.1
維生素B1	mg/100g	0.09	0.07	0.08	0.05	-
維生素B2	mg/100g	0.06	0.04	0.06	0.07	-
維生素B6	mg/100g	0.06	0.05	0.06	0.06	-
維生素C	mg/100g	108.8	107.9	171.2	76	87
維生素E	mg/100g	1.08	1.24	0.92	0.9	-
葉酸	μg/100g	200.2	208.4	167.2	72	121
菸鹼酸	mg/100g	0.41	0.23	0.42	0.03	ND

*1.花蓮一號、花蓮二號及花蓮3號營養成份依據2006本場委託財團法人食品工業發展研究所檢測。

2.大苦瓜營養成份參考（園藝之友第101期）依據日本五訂食品檢測成份。

3.野苦瓜營養成份檢測依據台南區農業改良場1998委請屏東科技大學農水產檢驗服務中心檢測。

表二、2006-2007 年 GAP 苦瓜第一期~第四期作栽培記錄

地點	期作	農戶姓名	地點、設施	種苗及來源
高雄	2006春夏	鍾壬亮	月美段3992號 水平棚架網室	花蓮1號，花蓮場94年春作採種 花蓮2號，花蓮場94年春作採種 小月，農友公司
花蓮	2006春夏	江玉寶	豐田段309號 塑膠布網室 水平棚架網室	花蓮1號，花蓮場94年春作採種 花蓮2號，花蓮場94年春作採種
雲林	2006春夏	廖俊凱	西螺新社段1299號 平架網架	花蓮1號，花蓮場94年春作採種 花蓮2號，花蓮場94年春作採種 小月，農友公司
新竹	2006春夏	陳阿發	光武段648號 塑膠布網室	花蓮1號，花蓮場94年春作採種 花蓮2號，花蓮場94年春作採種 小月，農友公司
高雄	2007 一期	鍾壬亮	月美段 水平棚架網室	花蓮 2 號，花蓮場 95 年夏作採種
花蓮	2007 一期	江玉寶	豐田段塑膠布網室 水平棚架網室	花蓮 2 號，花蓮場 95 年夏作採種
苗栗	2007 一期	陳阿發	興隆段 塑膠布網室	花蓮 2 號，花蓮場 95 年夏作採種
高雄	2007 二期	鍾壬亮	月美段 水平棚架網室	花蓮 2 號，花蓮場 95 年夏作採種
高雄	2007 三期	鍾壬亮	月美段 水平棚架網室	花蓮 2 號，花蓮場 96 年春作採種
花蓮	2007 三期	江玉寶	豐田段塑膠布網室	花蓮 2 號，花蓮場 96 年春作採種
苗栗	2007 三期	陳阿發	興隆段 塑膠布網室	花蓮 2 號，花蓮場 96 年春作採種
高雄	2007 四期	鍾壬亮	月美段 水平棚架網室	花蓮 2 號，花蓮場 96 年春作採種
高雄	2008 一期	鍾壬亮	月美段 水平棚架網室	花蓮 2 號，花蓮場 97 年春作採種 花蓮 4 號，花蓮場 97 年春作採種
花蓮	2008 一期	江玉寶	豐田段塑膠布網室 水平棚架網室	花蓮 2 號，花蓮場 97 年春作採種 花蓮 4 號，花蓮場 97 年春作採種
苗栗	2008 一期	陳阿發	興隆段 塑膠布網室	花蓮 2 號，花蓮場 97 年春作採種 花蓮 4 號，花蓮場 97 年春作採種
高雄	2008 二期	鍾壬亮	月美段 水平棚架網室	花蓮 2 號，花蓮場 97 年春作採種 花蓮 4 號，花蓮場 97 年春作採種

表二續、

種植日期	灌溉水源	有機肥種類廠牌	防治葯劑
2006.3.7	山泉水	豆餅、雞糞	病害→肉桂油 蟲害→菸草液
2006.3.3	山泉水	德城牌 燕子牌	病害→亞磷酸+海草精 蟲害→蘇力菌、草蛉
2006.3.8	地下水	牛糞有機肥	病害→水楊酸
2006.3.15	地下水	大地牌 苦茶柏	病害→菸草液
2007.3.1	山泉水	豆餅、雞糞	病害→肉桂油 蟲害→菸草液
2007.2.24	山泉水	德城牌 燕子牌	病害→亞磷酸+海草精 蟲害→蘇力菌、草蛉
2007.3.2	地下水	大地牌 苦茶柏	病害→菸草液
2007.4.24	山泉水	豆餅、雞糞	病害→肉桂油 蟲害→菸草液
2007.7.17	山泉水	豆餅、雞糞	病害→肉桂油 蟲害→菸草液
2007.7.6	山泉水	德城牌 燕子牌	病害→亞磷酸+海草精 蟲害→蘇力菌、草蛉
2007.7.16	地下水	大地牌 苦茶柏	病害→菸草液
2007.10.18	山泉水	豆餅、雞糞	病害→肉桂油 蟲害→菸草液
2008.2.26	山泉水	豆餅、雞糞	病害→肉桂油 蟲害→菸草液
2008.2.21	山泉水	德城牌 燕子牌	病害→亞磷酸+海草精 蟲害→蘇力菌、草蛉
2008.2.19	地下水	大地牌 苦茶柏	病害→菸草液
2008.5.6	山泉水	豆餅、雞糞	病害→肉桂油 蟲害→菸草液

表三、花蓮區農業改良場農化實驗室土壤樣品檢驗結果

地點	酸鹼度	有機質 (%)	磷 (ppm)	鉀 (ppm)	鈣 (ppm)	鎂 (ppm)	鐵 (ppm)
參考值	5.5-7.5	2-3	10-20	30-50	517-1142	48-96	11-24
（2006 春作）							
雲林	6.9	3.8	1188	77	3266	181	109
新竹	6.7	2.8	138	132	1149	141	136
高雄	4.8	0.7	150	119	452	56	390
花蓮	6.0	7.6	535	2130	1798	586	7
（2006 夏作）							
雲林	6.6	4.1	388	265	5682	185	86
新竹	5.8	4.5	225	148	3288	321	333
高雄	6.3	1.2	125	71	2485	158	1049
（2007 一期作）							
高雄	7.1	1.0	194	56	1131	105	846
苗栗	6.0	0.9	20	28	352	51	268
花蓮	7.0	0.5	131	20	5587	81	15
（2007 二期作）							
高雄	5.1	1.3	163	25	665	60	750
（2007 三期作）							
苗栗	4.4	1.8	58	55	278	27	360
花蓮	6.7	0.4	70	16	9899	144	2
高雄	6.1	1.0	75	150	1460	214	1069
（2007 四期作）							
高雄	5.9	0.1	163	22	305	24	231
（2008 一期作）							
高雄	6.7	1.6	938	88	841	110	311
苗栗	6.3	1.9	250	80	2257	80	105
花蓮	7.1	2.4	250	10	853	35	196
壽豐	7.4	1.2	175	51	7280	98	54

表三續、

地點	錳 (ppm)	銅 (ppm)	鋅 (ppm)	電導度 (ppm)	鎘 (ppm)	鉻 (ppm)	鎳 (ppm)	鉛 (ppm)
參考值	15-30	<20	<25	<0.5	<0.39	<10	<10	<15
（2006 春作）								
雲林	93	3.0	50.9	0.61	0.32	3.3	1.7	0.8
新竹	46	1.3	5.3	0.54	—	—	—	—
高雄	15	2.2	9.5	0.28	—	—	—	—
花蓮	34	0.6	10.1	4.97	—	—	—	—
（2006 夏作）								
雲林	148	2.6	45.8	1.35	-	-	-	-
新竹	19	18.5	24.7	0.50	0.16	0.7	2.1	1.3
高雄	54	6.9	9.5	0.22	0.16	1.1	0.8	2.6
（2007 一期作）								
高雄	84	4.2	7.7	0.19	0.30	1.7	0.7	5.8
苗栗	20	1.7	9.7	0.17	0.09	0.2	N.D.	N.D.
花蓮	79	0.4	1.8	0.15	0.04	0.03	0.1	0.9
（2007 二期作）								
高雄	27	3.7	6.7	0.31	0.07	0.5	0.2	N.D.
（2007 三期作）								
苗栗	71	1.9	8.8	0.68	0.07	0.2	N.D.	1.4
花蓮	106	N.D.	0.9	0.18	0.06	0.1	0.3	N.D.
高雄	58	5.6	11.2	0.16	N.D.	0.8	0.6	1.7
（2007 四期作）								
高雄	18	4.8	6.5	0.18	0.06	0.3	N.D	1.5
（2008 一期作）								
高雄	16	3.7	3.6	0.17	0.10	0.5	0.5	0.4
苗栗	39	0.6	4.4	0.70	0.08	N.D.	0.8	N.D.
花蓮	80	12.7	8.8	0.33	0.08	0.3	2.0	N.D.
壽豐	68	2.4	5.0	0.30	0.07	N.D.	0.04	1.18

表四、花蓮區農業改良場農化實驗室水體樣品檢驗結果

地點	酸鹼度	電導度 (mS/cm)	銅 (ppm)	鋅 (ppm)	鎘 (ppm)	鉻 (ppm)	鎳 (ppm)	鉛 (ppm)	錳 (ppm)
容許值	6.0-9.0	0.75	0.2	2.0	0.01	0.1	0.2	0.1	0.2
(2006)									
高雄	6.9	0.50	N.D.	N.D.	-	0.04	0.04	-	N.D.
西螺	6.7	2.02	N.D.	N.D.	-	0.02	0.06	-	0.87
新竹	7.4	0.56	N.D.	N.D.	-	0.03	0.08	-	N.D.
高雄	7.0	0.43	0.02	N.D.	-	0.004	0.01	-	N.D.
西螺	6.6	2.05	0.02	N.D.	-	0.006	N.D.	-	N.D.
(2007 一期作)									
高雄	6.2	0.41	N.D.	N.D.	0.004	0.02	N.D.	0.06	N.D.
苗栗	6.6	0.55	N.D.	N.D.	0.002	0.01	N.D.	N.D	N.D.
花蓮	7.7	0.43	0.03	N.D.	N.D.	N.D.	N.D.	N.D.	0.05
(2007 二期作)									
高雄 (1)	6.6	0.45	0.08	N.D.	0.020	0.00	0.01	N.D.	N.D.
高雄 (2)	6.8	0.43	0.054	N.D.	0.011	N.D.	0.01	N.D.	N.D.
(2007 三期作)									
苗栗	7.3	0.56	0.1	N.D.	0.005	0.02	N.D.	N.D.	0.13
花蓮	6.7	0.45	0.1	N.D.	0.002	0.02	N.D.	N.D.	N.D.
高雄	7.0	0.18	0.1	N.D.	0.008	0.01	N.D.	N.D.	N.D.
(2007 四期作)									
高雄	5.9	0.37	N.D.	N.D.	0.005	N.D.	0.03	N.D.	N.D.
(2008 一期作)									
高雄	7.0	0.45	N.D.	N.D.	0.009	0.03	0.06	N.D.	N.D.
苗栗	7.1	0.54	N.D.	N.D.	0.006	0.01	0.04	N.D.	N.D.
花蓮	7.2	0.29	N.D.	N.D.	0.008	N.D.	0.04	N.D.	N.D.

表四、花蓮區農業改良場農化實驗室水體樣品檢驗結果

項目	日期/地點	酸鹼度	有機質(%)	氮(%)	磷(%)	鉀(%)	鈣(%)	鎂(%)	鐵(ppm)
（2006 春作）									
農華牌	西螺	5.7	73.3	4.5	1.0	1.1	1.9	0.6	5766
大地牌	新竹	5.1	79.6	5.2	1.6	2.3	3.0	0.5	1813
（2006 年夏作）									
油車堆肥	西螺	5.4	48.0	5.6	1.0	0.4	-	2.6	0.3
牛糞		9.3	19.9	1.7	0.2	0.2	-	2.9	960
（2007）									
有機肥（德城 33）	3.14 壽豐	7.9	26.0	1.7	0.2	0.5	1.4	1.3	6092
有機肥	7.19 高雄	6.5	70.8	3.7	2.0	0	0.002	0	1347
有機肥	10.24 高雄	5.3	93.7	5.6	0.6	1.4	1.3	0.3	1
（2008 春作）									
有機肥（勇將）	4.10 玉里	7.4	75.3	6.0	1.4	1.6	0.5	0.5	1
有機肥（金錢 6 號）	4.10 玉里	8.4	79.1	4.6	0.9	1.2	0.9	1.1	1

表五續、

項目	日期/地點	錳 (ppm)	銅 (ppm)	鋅 (ppm)	電導度 (mS/cm)	鎘 (ppm)	鉻 (ppm)	鎳 (ppm)	鉛 (ppm)
（2006 春作）									
農華牌	西螺	277	40.5	151.7	6.54	0.23	5.88	2.49	2.7
大地牌	新竹	953	45.0	146.8	7.84	0.83	12.49	4.61	N.D.
（2006 夏作）									
油車堆肥	西螺	218	11	6.5	7.09	-	-	-	-
牛糞		33	5.2	23.7	7.98	-	-	-	-
（2007）									
有機肥（德城 33）	3.14 壽豐	124	4.8	30.8	0.85	3.936	104.85	228.95	9.344
有機肥	7.19 高雄	198	22.4	168.7	6.34	0.51	3.86	3.67	2.23
有機肥	10.24 高雄	6	0.2	0.2	4.49	0.129	N.D.	0.298	0.099
（2008 春作）									
有機肥（勇將）	4.10 玉里	4	0.2	0.2	2.97	0.074	N.D.	0.088	0.015
有機肥（金錢 6 號）	4.10 玉里	4	0.8	8.5	1.88	0.02	0.608	0.492	0.342

表六、2006-2007 年度 GAP 量產苦瓜產量調查

地點	品系	耕作方式	小區產量 公斤/公尺^2	公頃產量 (公噸/公頃)	價格 (元/公斤)	產值 (仟元/公頃)	採收期
（2006 春作）							
花蓮	花蓮 1 號		1538/1494	10.7	90	963	5.8-7.5
	花蓮 2 號		1022/1162	8.7	60	522	5.8-7.5
高雄	花蓮 1 號		662.2/572	11.6	24	278	5.4-6.4
	花蓮 2 號		315/352	8.9	21	187	5.4-6.4
	小月(對照)		246/352	7.0	36	252	5.4-6.4
雲林	花蓮 1 號		745/341	35.9	60	2,152	5.10-6.27
	花蓮 2 號		531.5/170.5	51.4	60	3,084	5.10-6.27
	小月(對照)		267.5/170.5	25.5	60	1,529	5.10-6.27
新竹	花蓮 1 號		482.2/129.3	29.2	45	1,314	5.10-6.27
	花蓮 2 號		928.2/258.6	24.1	45	1,080	5.10-6.27
	小月(對照)		141.2/129.3	10.9	45	4,914	5.10-6.27
（2006 夏作）							
花蓮	花蓮 1 號		78.3/131	6.0	90	540	10.5-11.15
	花蓮 2 號		74/131	5.6	60	336	9.20-11.13
	小月(對照)		72.5/131	5.5	90	495	9.20-11.13
高雄	花蓮 1 號		250.1/216	11.6	25	290	9.9-12.20
	花蓮 2 號		323.5/216	15.0	25	375	9.5-12.26
雲林	花蓮 1 號		151.2/131	11.5	60	690	9.14-10.11
	花蓮 2 號		184.8/131	14.1	60	846	9.7-10.12
	小月(對照)		63.5/131	4.8	60	288	9.7-10.12
新竹	花蓮 1 號		261.8/258.6	10.1	45	455	9.9-11.16
	花蓮 2 號		548.4/388	14.1	45	634500	9.5-11.16
（2007 一期作）							
花蓮	花蓮 2 號	輪作	1387/320	43.4	60	2604.0	5.1~7.31
高雄	花蓮 2 號	輪作	1994/310	61.0	51.1	3117.1	5.1~8.13
苗栗	花蓮 2 號	新地	2112/360	58.7	50	1935	5.21~7.16
（2007 二期作）							
高雄	花蓮 2 號	連作	556/385	21.4	15.6	333.8	6.8~8.6
（2007 三期作）（柯羅莎颱風 10 月 4-6 日）							
花蓮	花蓮 2 號	輪作	108/330	3.52	60	211.2	9.15~10.5
高雄	花蓮 2 號	輪作	123/380	4.7	32	150.4	9.17~10.5
苗栗	花蓮 2 號	輪作	535/380	20.1	50	1005	5.9~12.11
（2007 四期作）							
高雄	花蓮 2 號	連作	334/600	20.0	51.4	1028	12.22~1.28

表七、苦瓜 GAP 生產各試區果實性狀

地點	品系	果色	果重 (g)	果長 (mm)	果寬 (mm)	調查日期
（2006 夏作）						
花蓮	花蓮 1 號	綠	122.1	127.6	43.6	10.15
	花蓮 2 號	白	120.2	153.4	55.3	10.15
高雄	花蓮 1 號	綠	138.3	136.8	54.2	10.12
	花蓮 2 號	白	171.7	176.2	56.2	10.12
	小月(對照)	深綠	57.2	97.8	42.5	10.12
雲林	花蓮 1 號	綠	98.2	123.9	46.7	10.13
	花蓮 2 號	白	135.6	170.2	52.2	10.13
	小月(對照)	深綠	66.9	115.6	46.8	10.13
新竹	花蓮 1 號	綠	87.9	89.1	48.0	10.13
	花蓮 2 號	白	192.7	175.6	61.1	10.13
	小月(對照)	深綠	55.1	116.9	43.3	10.13
（2007 一期作）						
花蓮	花蓮 2 號	白	161.75	162	57.7	6.8
高雄	花蓮 2 號	白	199.0	185.5	59.20	6.7
苗栗	花蓮 2 號	白	148.03	168.9	57.6	7.16
（2007 二期作）						
高雄	花蓮 2 號	白	138.0	153	56.16	6.7
（2007 三期作）						
花蓮	花蓮 2 號	白	-	-	-	-
高雄	花蓮 2 號	白	112.3	132	56.5	10.18
苗栗	花蓮 2 號	白	136.92	156	57.37	9.12
（2007 四期作）						
高雄	花蓮 2 號	白	215.9	297.9	66.7	12.19

表八、2006-2007 年 1-12 月苦瓜 GAP 各試驗點平均溫度(℃)

地點	花蓮		新竹	苗栗	雲林	高雄	
月份	2006	2007	2006	2007	2006	2006	2007
1	18.9	18.5	15.5	16.3	17.1	20.5	19.7
2	18.8	20.2	15.9	17.7	17.8	21.5	22.1
3	19.5	20.8	18.0	19.1	19.3	22.6	23.5
4	22.8	21.9	22.5	20.8	24.0	25.9	25.0
5	25.1	25.4	24.5	25.7	25.9	27.7	27.7
6	25.9	27.7	27.0	27.6	27.0	28.5	28.9
7	27.8	29.8	28.6	30.0	28.4	28.4	30.2
8	27.1	27.6	28.1	28.4	28.1	28.4	28.1
9	25.4	27.0	25.8	27.6	26.8	27.1	28.2
10	24.0	25.0	23.6	24.5	25.3	26.6	26.8
11	21.6	22.1	21.0	20.5	22.7	24.9	23.4
12	18.7	20.5	16.9	18.6	18.2	21.5	21.8

苦瓜GAP生產栽培曆制定

2006-2007 年試驗期間調查之各試驗地點全年每月溫度資料，包括花蓮、雲林、新竹、苗栗及高雄（表八），制定各試驗點之生產栽培曆如圖二，其中花蓮地區 2006 年 3 月 19.5℃，2007 年 2 月氣溫已達攝氏 20 度，是育苗適合時期；雲林 4 月溫度達 24℃，2007 年苗栗地區 4 月氣溫才達攝氏 20 度以上，是育苗適合時期；以上 2 處皆在 3 月定植，4 月開花，植株逐漸生育旺盛，5 月初進入採收期，採收至 6 月底，果實生長良好（圖三)。高雄地區 2006 年全年平均氣溫皆達攝氏 20 度以上 2007 年 1 月有 19.7℃，即表示全年皆適合育苗，在 2-3 月進行一期作定植，其開花期最早。二期作 4 月進行，三期作 7 月進行，四期作 10 月進行；花蓮、雲林、新竹及苗栗等地點之 12 月氣溫即降至攝氏 20 度左右，苦瓜開花結果皆受影響，因此此處之栽培期適合在 3 月-11 月，而高雄地區全年平均氣溫皆達攝氏 20 度以上，12 月份生產之果實品質及外觀皆正常（圖四)，是可以全年栽培。

結語

經過雜交育種方法育成之基質穩定，生長勢強，產量高的山苦瓜一代

雜交品種爲材料，建立苦瓜適合作爲保健食品或中草藥材材料之良好作業規範（GAP）生產標準作業流程，可提供未來產業界進行藥用作物藥效成分評估與開發藥用作物成爲保健食品的指標。苦瓜不同品種、不同栽培地點、不同期作生產材料經過農藥殘留檢測，配合台灣大學，慈濟大學及中國醫藥大學相關實驗室進行 PPAR 活性檢測、抗癌活性檢測等，其結果顯示不同品種或不同栽培地點或不同期作之生產材料具有不同程度之差異性，足見生產苦瓜作爲中藥材標準操作規範建立之重要性。未來需更進一步探討最適當之生產過程，以生產最好的材料，以增加試驗正確之再現性，提高保健苦瓜之價值。

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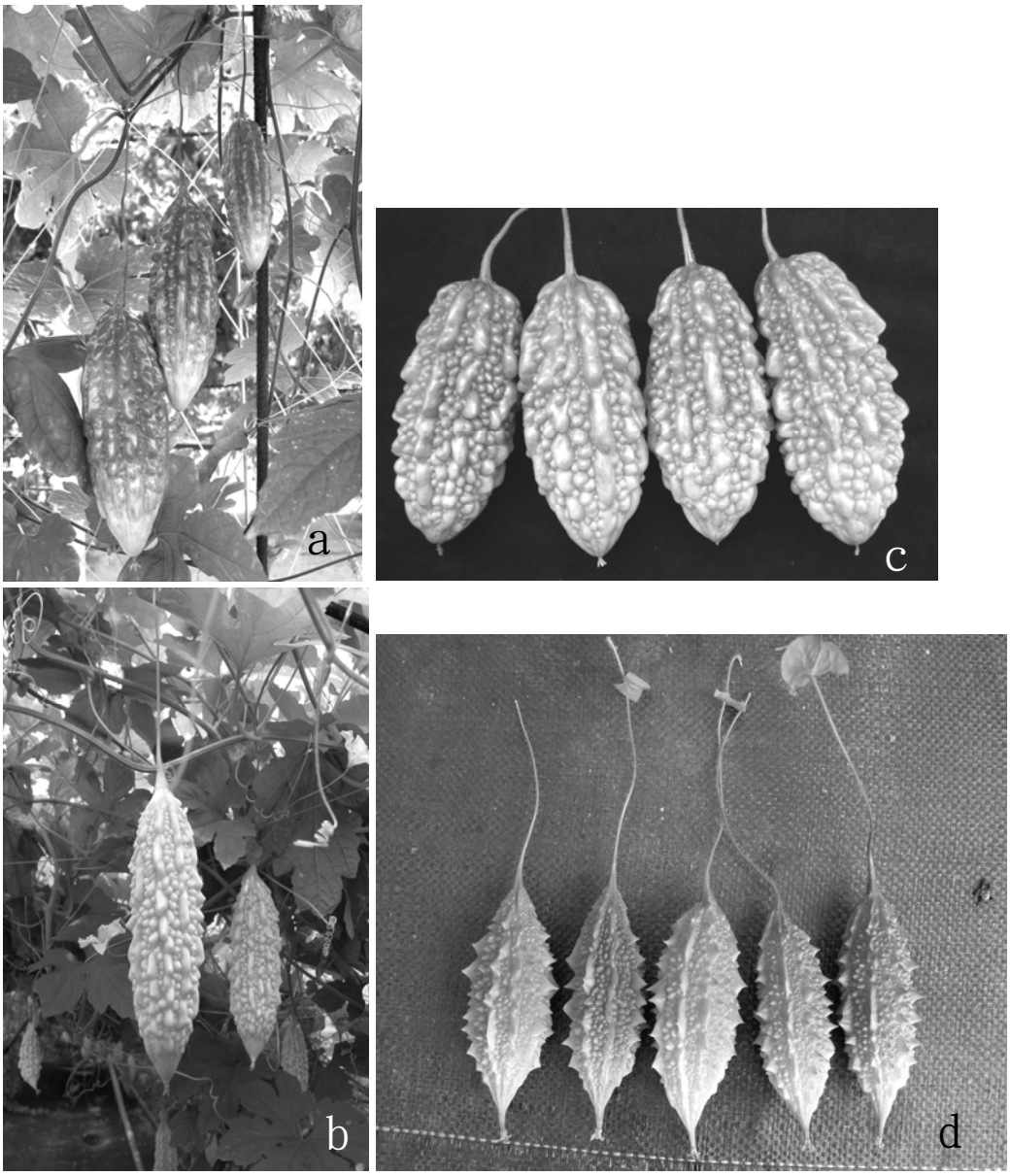
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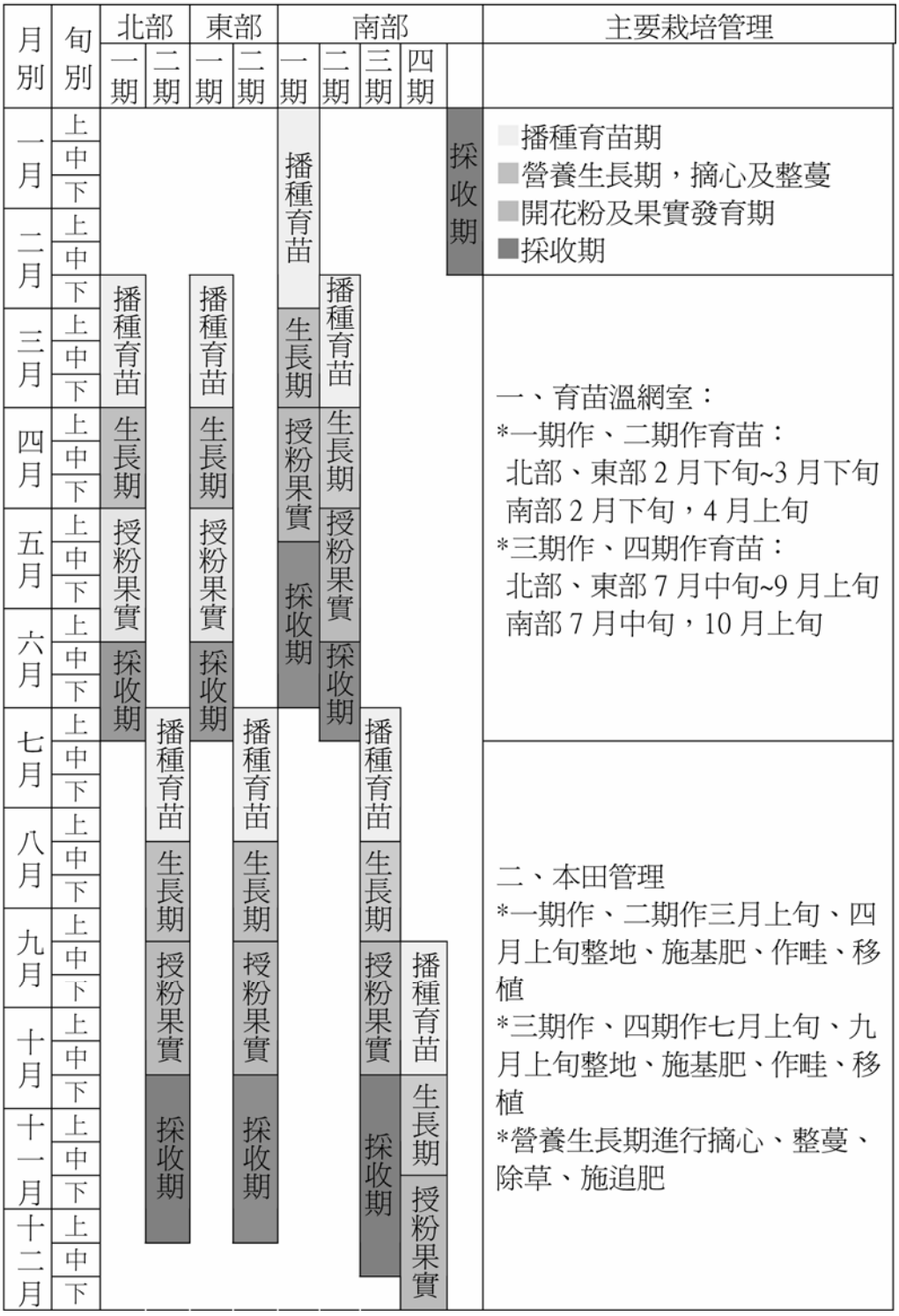
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圖一、苦瓜新品種 a 花蓮 1 號、b 花蓮 2 號
c 花蓮 3 號、d 花蓮 4 號

圖二、苦瓜在臺灣北東南試區之栽培曆





圖三、2006 年新竹試區苦瓜 GAP 生產植株生育情形



圖四、2007 花蓮試區苦瓜 GAP 生產植株生育情形（花蓮二號）

Breeding of New Bitter Gourd Varity and establishment of the GAP System

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ABSTRACT

By using the cross breeding approaches between wild and native bitter gourds, the Hualien District Agricultural Research and Extension Station (Hualien DARES) have successfully bred four new cultivars, Hualien No.1', Hualien No.2', Hualien No.3' and Hualien No.4' during 2006-2008. Following the "Good Agricultural Practice" (GAP) cultivating techniques, these new varieties were grown and provided for further bioactivity assays and relative studies. To establish the GAP system, Halien No.1' and Hualine No.2' were conducted in a one-year-two-crop trial at four areas including Hsinchu, Yunlin, Kaohsiung and Hualien during 2006; and cultivars Hualien No.2' and Hualien No.4' were conducted in both a two-year-two-crop trial and a two-year-four-crop trial in Miaoli and Hualien; and in Kaohsiung, respectively during 2007-2008. Special farmer and farm has been selected for the organic farming. Following the GAP principles, we have detected the secure level of soil, fertilizer and irrigate water at regular intervals. No detectable difference of fruit shape, fruit color and full-size has been observed among the whole planting experiment.This indicates the stable agriculture properties of these new cultivars.The highest yield for spring crop is observed in the Kaohsiung area of 61 tons/ha. According to data recorded from the growth conduction, the growth yield and the climate condition, we recommended the best planting period for the north, east and central areas is from March through October.

However, there have no boundaries of planting period for the south area (covering the whole year).

Key words：bitter gourd, GAP (Good Agricultural Practice)

由苦瓜中篩選可改善胰島素抗性之活性天然物

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摘　要

第二型糖尿病發生的機制目前認爲與細胞胰島素抗性（insulin resistance）的形成有關。因此目前醫學界認爲若能治療胰島素抗性，將能預防第二型糖尿病的發生或改善已患該病者之病情。本研究模仿動物體內胰島素抗性發生的機制，測試以 TNF-α（tumor necrosis factor-α）刺激，誘導小鼠 FL83B 肝臟細胞產生胰島素抗性，以建立一糖尿病藥物篩選平台，並利用此平台進行苦瓜降血糖活性成分之篩選。以此平台檢測白蓮苦瓜各部份之甲醇粗萃物，發現白蓮苦瓜莖材、果實、種子之粗萃物，皆有改善細胞胰島素抗性、促進葡萄糖吸收之活性。將白蓮苦瓜莖材之甲醇粗萃物進一步萃取、分層，發現其中正己烷層具有活性。再將正己烷層進一步分成 22 個分液（fractions），發現其中 3 個分液有改善細胞胰島素抗性的降血糖活性。分液 13 及分液 18 之成分已被純化鑑定。其中，純物質 CH10、CH63 及 CH93 被證實對胰島素抗性細胞的葡萄糖吸收及 IRS-1 的酪胺酸磷酸化均有明顯提高作用。同時，這些物質亦被證明可活化 AMP-activated protein kinase。以相同的方法對新品種山苦瓜，花蓮 2 號，進行篩選工作，目前至少發現了一種三萜類化合物具有相同的活性。這些結果建議苦瓜含有改善胰島素抗性的成分，具有開發爲第二型糖尿病保健食品或治療藥物之潛力。

關鍵詞：第二型糖尿病、胰島素抗性、白蓮苦瓜、花蓮 2 號山苦瓜、AMP-activated protein kinase, FL83B 細胞

前言

糖尿病是目前世界各國最普遍的多病因代謝疾病（Gerich, 2001）。台灣糖尿病人口不僅逐年增加，且爲十大死亡原因排名第四位。台灣地區的糖尿病型態以非胰島素依賴型糖尿病爲主，亦即第二型糖尿病，約佔罹患率的 95%左右。根據統計資料顯示，罹患有第二型糖尿病者，在患病之前身體會先出現胰島素抗性（又稱代謝症候群），而此症狀若沒有及時治療或改善，將來可能就會發展成爲第二型糖尿病，亦即胰島素抗性症狀患者爲罹患第二型糖尿病的高危險群。因此目前認爲若能對此高危險群者採取預防措施，盡快矯正其胰島素抗性的問題，則可預防將來繼續發展爲糖尿病，並能節省醫療資源。

世界各地的民俗草藥治療糖尿病已有十分悠久的歷史。根據本草綱目的記載及民間流傳，葫蘆科植物包括苦瓜、南瓜及冬瓜等食用植物，經常食用有降血糖之功效。然而這些草藥雖民間流傳爲有效用，卻往往因爲缺乏系統性的研究與比較，所以仍停留於民間草藥或秘方，實在可惜。以苦瓜爲例，於中國、日本、印度、南美洲等地，苦瓜不僅長期被使用爲食用性蔬菜，亦被使用爲傳統民俗藥物，包括用於治療糖尿病（Grover and Yadav, 2004），但是其降血糖之天然成分卻一直不清楚，且沒有被系統性地分析與研究。缺乏一適當的分析、篩選工具是主要原因之一。

植物品系眾多，且所含天然物相當複雜，若欲分析其活性成分，所需進行的篩選工作相當龐大，無法直接以實驗動物如老鼠等進行篩選工作。因此，本研究開發以細胞爲工具，將細胞株誘導使具有胰島素抗性，以此爲一平台篩選出可克服細胞胰島素抗性、促進細胞葡萄糖吸收的活性天然物。

胰島素抗性是指血液循環中之胰島素調節體內能量代謝的能力降低(Zick, 2001)。亦即，在正常濃度的胰島素生理濃度下，身體細胞對胰島素的敏感度下降甚或對之毫無反應。研究資料顯示，除了糖尿病，胰島素抗性也會導致其他代謝相關的疾病，包括高血壓(hypertension)、血脂異常(dyslipidemia)、動脈硬化(atherosclerosis)、微蛋白尿(microalbuminuria)、血液易凝固(hypercoagulability)、中央性肥胖(central obesity)及其他心血管疾病等(Weyer et al., 1999; Isomaa et al., 2001; Hanley et al., 2002)，因此胰島素抗性又被稱爲代謝症候群(the metabolic syndrome) (Lebovitz, 2001)。研究資料指出，第二型糖尿病患者在患病前之 10-20 年間會先出現胰島素抗性，

而且第二型糖尿病患者皆患有胰島素抗性(Lillioja, 1988)。因此胰島素抗性的出現被認爲是一個個體可能會發展成第二型糖尿病之先兆(Lillioja, 1988; Warram et al., 1990; Shulman, 2000)。研究證實，胰島素抗性之分子機制與胰島素訊息傳遞路徑中之 IRS(insulin receptor substrate)的酪胺酸磷酸化(tyrosine phosphorylation)有關。胰島素訊息傳遞之過程，IRS 因接受酪胺酸磷酸化而被活化，進而繼續活化下游之胰島素訊息傳遞路徑。但證據顯示，具有胰島素抗性的細胞，其 IRS 的酪胺酸磷酸化受到干擾，所以無法活化下游的路徑，使胰島素訊息傳遞路徑之活動中斷(Dresner et al., 1999; Lebovitz and Banerji, 2004)。

體重過重的患者是罹患第二型糖尿病的高危險群。研究其機制，發現可能與脂肪細胞所分泌的 adipokines 有關。例如，研究發現，肥胖者的脂肪細胞會分泌較多 TNF-α，而 TNF-α 會抑制 IRS-1（爲 IRS 的主要 isoform 之一）的酪胺酸磷酸化而導致胰島素抗性的產生(Zick, 2001)。此時，若不及時治療，最終即可能形成第二型糖尿病。故本研究意圖發展一個篩選系統，以具有胰島素抗性的細胞爲平台，篩選出可改善或克服細胞胰島素抗性的物質，幫助預防或改善第二型糖尿病。

胰島素於動物體內主要作用於肝臟細胞、肌肉細胞及脂肪細胞。已有實驗證實以 TNF-α 處理肌肉細胞株(del Aguila et al., 1999)及脂肪細胞株(Iwata et al., 2001)，會使細胞產生胰島素抗性。但是常用的肌肉細胞株及脂肪細胞株，如 C2C12 及 3T3L1，皆須先經過分化才具有胰島素敏感性。如此，增加實驗操作的複雜度，使之要發展成爲一個需要篩選大量物質的篩選系統較不利。而且不同盤或不同批次的細胞之間，分化的程度可能不相同，使細胞對胰島素的敏感程度不同，造成實驗誤差，使不同物質之間效果的比較會有差異，降低篩選工作的準確性。因此，本研究另外發展以肝臟細胞株作爲篩選平台的方法。所選用之細胞株爲小鼠正常肝臟細胞株 FL83B。此細胞株不必經過分化就有胰島素敏感性。本研究更發現 FL83B 細胞株經過 TNF-α 處理亦會產生胰島素抗性。目前已利用此平台由白蓮苦瓜及花蓮 2 號山苦瓜中篩選出數種可克服細胞胰島素抗性的活性天然物。並對這些天然物之作用機轉進行初步的探討。

內容

一、以TNF-α 誘導FL83B細胞產生胰島素抗性

將FL83B以不同濃度之TNF-α(10 ng/ml、20 ng/m)處理細胞5小時，接著再培養於無血清培養基2小時，最後再改用MEM培養基加100 nM胰島素，刺激細胞的葡萄糖吸收作用。分別於加入胰島素後第0、1、2、3、4、5小時取30μl培養基檢驗其葡萄糖濃度。如圖1所示。X軸代表加入胰島素後的時間，Y軸之500 nm吸光值與葡萄糖濃度成正比，吸光值愈高代表葡萄糖濃度愈高。Control爲正常的FL83B細胞沒有胰島素刺激；control+insulin爲接受胰島素刺激的FL83B細胞；TNF-α指經TNF-α處理5小時後再添加胰島素刺激的細胞。資料顯示，control+insulin與control相較，果然其培養基中之葡萄糖濃度有更明顯的減少，顯示FL83B細胞具有胰島素敏感性，在胰島素刺激下葡萄糖吸收速率明顯增加，但以10 ng/ml或20 ng/ml的TNF-α處理過的FL83B細胞，雖以胰島素刺激之，其培養基中葡萄糖濃度的降低明顯趨緩，其葡萄糖吸收速率接近control，暗示這些細胞對胰島素的敏感性降低，亦即細胞產生胰島素抗性。這些結果顯示以10 ng/ml或20 ng/ml的TNF-α進行前處理，均能誘導FL83B細胞產生胰島素抗性，其中以20 ng/ml的TNF-α具有更明顯的誘導效果。

二、檢驗白蓮苦瓜萃取物對胰島素抗性細胞葡萄糖吸收之影響

將分別來自白蓮苦瓜之果實、種子、莖部的甲醇萃取物，處理上述具有胰島素抗性的FL83B細胞，再檢驗這些細胞的葡萄糖吸收。結果如圖2所示[resistant+insulin爲胰島素抗性細胞（經20 ng/ml TNF-α處理之FL83B細胞）接受胰島素刺激]，白蓮苦瓜果實、種子、莖材萃取物之效果與50 μM troglitazone（爲一胰島素增敏劑，臨床上用以治療胰島素抗性，這裡作爲陽性對照組）的效果相似，均具有促進胰島素抗性細胞葡萄糖吸收的能力 ($p < 0.05$ versus resistant+insulin)，顯示具有改善細胞胰島素抗性之效果。

將白蓮苦瓜莖材的甲醇萃取物進一步分層萃取（如圖3所示），再一一檢驗各層之活性。如圖4所示，100 μg/ml甲醇粗萃物及10 μg/ml正己烷層[hexane；圖3中標示爲(2)者]萃取物具有促進胰島素抗性細胞葡萄糖吸收的效果，顯示具有改善胰島素抗性之功效。其餘的萃取層，有的沒有明顯差異，有的則甚至較resistant+insulin的葡萄糖吸收速率還慢[100 μg/ml乙酸乙酯層，圖3中標示爲(3)者；100 μg/ml正丁醇層，圖3中標示爲(4)者]，暗示不但沒有改善胰島素抗性之功效，且對葡萄糖吸收可能有抑制效果。

接著將正己烷層萃取物進一步分爲22個分液(fractions；如圖3所示)，並一一檢驗這些分液的活性。結果發現分液13、18及22之活性最高、最明顯。將分析分液10~22之結果分示於圖5A~C。

三、檢驗白蓮苦瓜單一天然物之活性及初步分子機制之探討

將分液13及18進一步純化。由分液13共獲得7種化合物，皆爲三萜類(triterpenes)；由分液18共獲得5種化合物，一種三萜類，三種固醇類(steroids)，一種芳香類化合物。分液13所含的最主要兩種化合物分別爲(23E)-cucurbita-5,23,25-triene-3β,7β-diol (以下簡稱爲CH10) 及3β,25-dihydroxy-7β-methoxycucurbita-5,23(E)-diene (以下簡稱爲CH63)。分液18所含的最主要化合物是 3β,7β,25-trihydroxycucurbita-5,23(E)-dien-19-al (以下簡稱爲 CH93) ，是一種三萜類化合物。三者的結構如圖5D所示。將三者分別以胰島素抗性細胞測試，發現皆可克服胰島素抗性而促進細胞葡萄糖吸收（圖6A）。

將CH10、CH63及CH93所處理過之胰島素抗性細胞分析其IRS-1酪胺酸磷酸化情形，如圖6B所顯示，三者皆使胰島素抗性細胞內之IRS-1酪胺酸磷酸化提高，顯示三者可活化IRS-1。再者，分析亦發現，此三種化合物皆能於胰島素抗性細胞內活化AMPK (AMP-activated protein kinase)（如圖6C所示）。因爲前人已證實AMPK可提高IRS-1之酪胺酸磷酸化而活化胰島素訊息傳遞路徑(Jakobsen et al., 2001)，所以，這些結果暗示，CH10、CH63及CH93可能是經由活化AMPK而提昇IRS-1之酪胺酸磷酸化，進而活化胰島素訊息傳遞路徑，所以可以克服細胞的胰島素抗性而改善細胞對胞外葡萄糖的吸收。目前這三種天然物之詳細作用機制正積極探討中。

四、檢驗花蓮2號山苦瓜單一天然物的活性

以與上述相似的方法對新品種苦瓜，花蓮2號山苦瓜，進行活性天然物之篩選。於過程中發現有數個分液具有活性，包括分液16。目前分液16已被進一步純化而得到3個化合物，分別簡稱爲RA2-10、RA2-11及RA2-19，皆爲三萜類化合物。將三者分別以胰島素抗性細胞測試，發現RA2-19可克服胰島素抗性而促進細胞葡萄糖吸收，但是另外兩種化合物的活性則較不明顯（圖7）。所以，目前亦正積極探討RA2-19之作用機制。

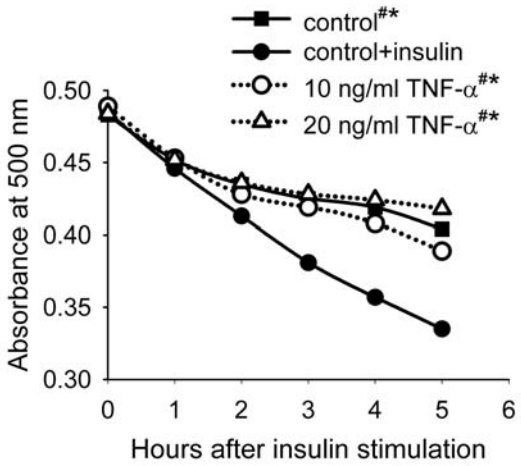


圖 1. 以 TNF-α 誘導 FL83B 細胞產生胰島素抗性。Control 爲正常的 FL83B 細胞沒有胰島素刺激；control+insulin 爲正常 FL83B 細胞受胰島素刺激；TNF-α 代表經 TNF-α 處理後再添加胰島素刺激的細胞。所顯示結果爲三次重複實驗之平均值±標準差(mean±SD)。所有資料皆以 two-way ANOVA 與“control+insulin”分析比較。#代表 p between groups < 0.05; *代表 p of interaction < 0.05.

Fig. 1. Induction of insulin resistance in FL83B cells by TNF-α. Control was normal cells without insulin stimulation; control+insulin was normal cells stimulated with insulin; other groups of cells were pre-incubated for 5 hours in medium containing either 10 ng/ml or 20 ng/ml of TNF-α, then stimulated with insulin. Glucose concentrations of the media were assayed at the time points indicated on the x-axis. Glucose concentrations were presented as absorbance at 500 nm and plotted as the y-axis. Each experiment was carried out in triplets. Data represent mean ± SD. Dataset of each cell group was statistically analyzed versus that of “control+insulin”by two-way ANOVA. $^{\#}p$ between groups < 0.05; * p of interaction < 0.05.

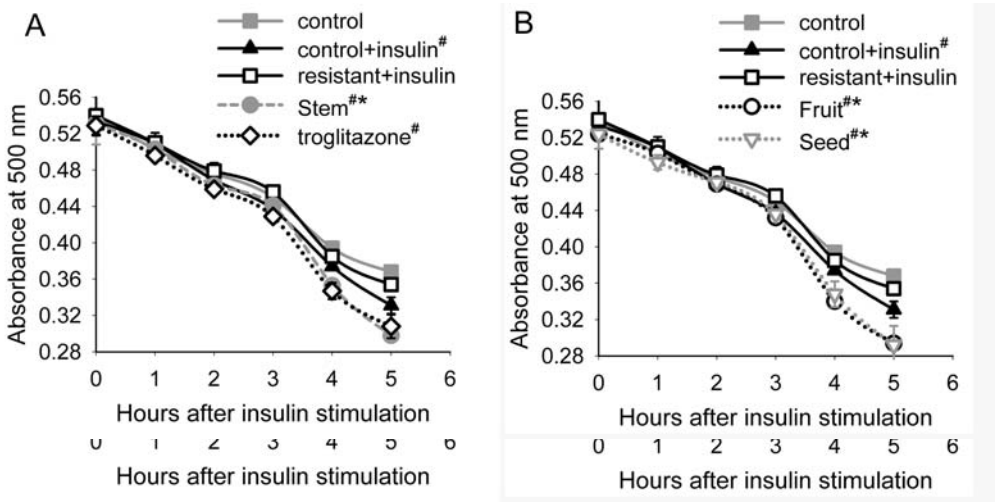


圖 2. 分析白蓮苦瓜各部甲醇萃取物對具胰島素抗性之 FL83B 細胞葡萄糖吸收之影響。Control 與 control+insulin 的意義與圖 1 同。Resistant+insulin 爲以 20 ng/ml TNF-α 處理過之胰島素抗性細胞，以胰島素刺激之。其他組細胞則是分別以 50μg/ml 白蓮苦瓜之果實（fruit）、種子（seed）、或莖部（stem）的甲醇萃取物處理胰島素抗性細胞，同時以胰島素刺激之。所顯示結果爲二重複實驗之平均值±標準差（mean±SD）。資料皆以 two-way ANOVA 與“resistant +insulin”分析比較。#代表 p between groups < 0.05; *代表 p of interaction < 0.05.

Fig. 2. Glucose uptake assays for cells treated with the crude extracts from different parts of *M. charantia*. Control and control+insulin were identical to those in Figure 1. Resistant+insulin was cells incubated in 20 ng/ml of TNF-αfor 5 hours (i.e. insulin-resistant cells) before stimulated by insulin. Other groups of cells were insulin-resistant cells treated with 50μg/ml of the methanol extract of either the stem, fruit, or seed of *M. charantia*, or treated with 50μM troglitazone, then stimulated by insulin. All groups of cells were assayed simultaneously, but due to the overlapping of curves, crude extract-treated groups were presented separately in two figures to make their curves more observable. The assays were carried out in duplicates. Data represent mean ± SD. Dataset of each cell group was statistically analyzed versus that of “resistant+insulin”by two-way ANOVA. #p between groups < 0.05; * p of interaction < 0.05.

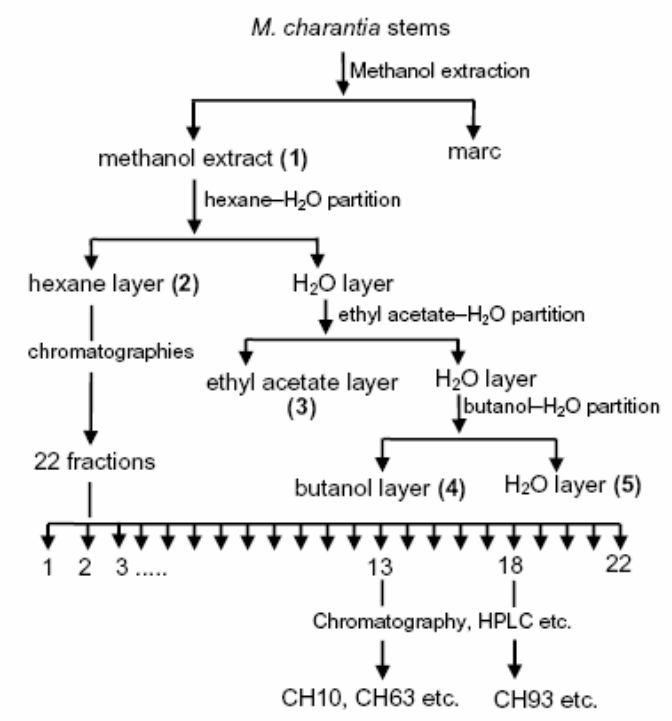


圖 3. 白蓮苦瓜莖材萃取物分離、純化流程圖。

Fig. 3. A flow chart showing the partitioning and purification of components in the methanol extract of the stem of *M. charantia.*

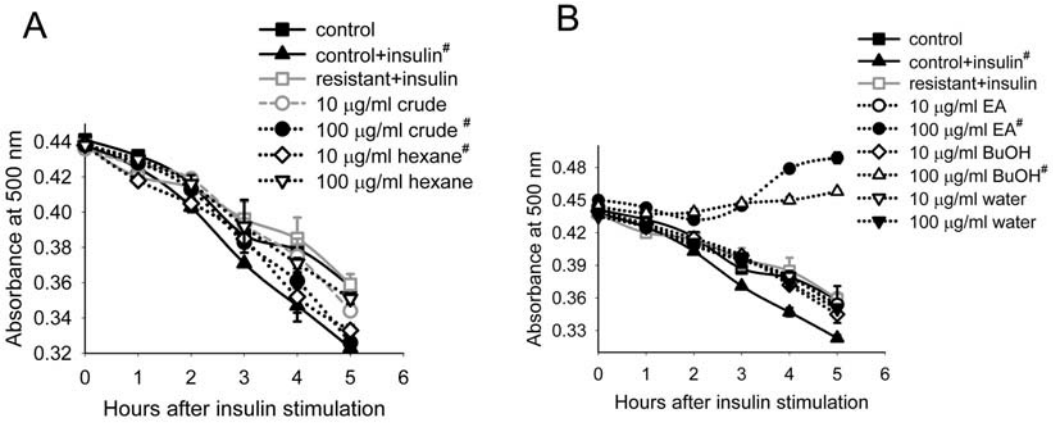


圖 4. 分析白蓮苦瓜莖材各萃取層對具胰島素抗性之 FL83B 細胞葡萄糖吸收之影響。Control、control+insulin、resistant+insulin 的意義與圖 2 同。其他組細胞則是分別以 10 或 100μg/ml 白蓮苦瓜莖部甲醇粗萃物（crude）、正己烷層（hexane）、乙酸乙酯層（EA）、正丁醇層（BuOH）、水層(water)處理胰島素抗性細胞，同時以胰島素刺激之。所顯示結果爲三重複實驗之 mean±SD。資料皆以 two-way ANOVA 與“resistant +insulin”分析比較。#代表 p between groups < 0.05 及 p of interaction < 0.05.

Fig. 4. Glucose uptake assays for cells treated with the partitions of the methanol extract of bitter melon stems. Glucose uptake assays were performed as in Figure 2. Crude, hexane, EA (ethyl acetate), BuOH (butanol), and water represent insulin-resistant cells treated with the partition marked as (1), (2), (3), (4), and (5) in Figure 3, respectively, then stimulated with insulin. The assays were carried out in triplets. Data represent mean ± SD. Dataset of each cell group was statistically analyzed versus that of “resistant+insulin”by two-way ANOVA. #p between groups < 0.05 and p of interaction < 0.05.

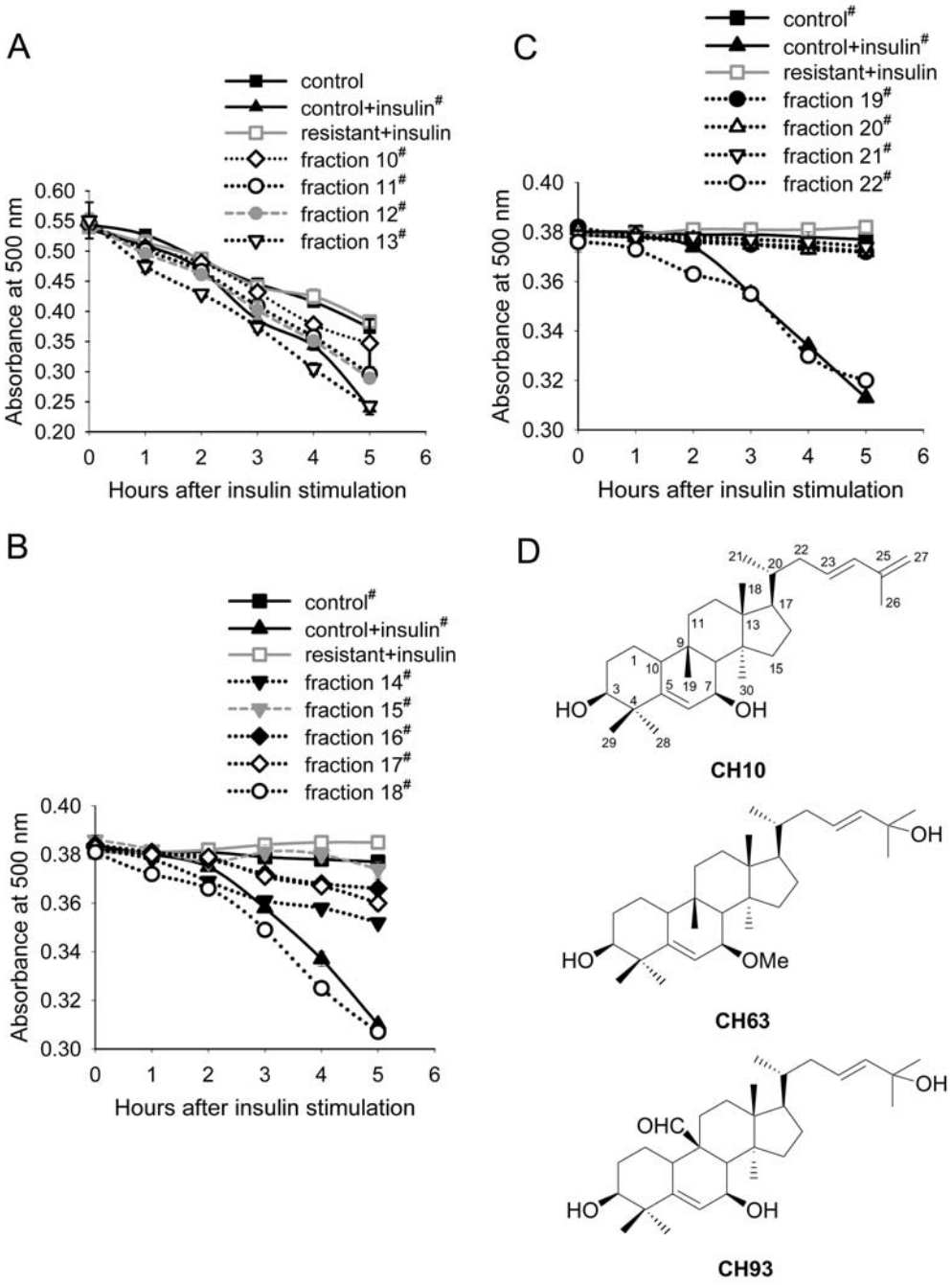

A
control
control+insulin#
resistant+insulin
fraction 10#
fraction 11#
fraction 12#
fraction 13#
Absorbance at 500 nm
Hours after insulin stimulation
B
control#
control+insulin#
resistant+insulin
fraction 14#
fraction 15#
fraction 16#
fraction 17#
fraction 18#
Absorbance at 500 nm
Hours after insulin stimulation
C
control#
control+insulin#
resistant+insulin
fraction 19#
fraction 20#
fraction 21#
fraction 22#
Absorbance at 500 nm
Hours after insulin stimulation
D
CH10
CH63
CH93

圖 5. 分析白蓮苦瓜正己烷層各分液對胰島素抗性細胞葡萄糖吸收之影響響。以 10 μ g/ml 各分液處理胰島素抗性細胞，再分析其葡萄糖吸收情形。分液 10~22 的結果分示於 A~C。所顯示結果爲三重複實驗之 mean±SD。資料皆以 two-way ANOVA 與“resistant +insulin”分析比較。#代表 p between groups < 0.05 及 p of interaction < 0.05. D, CH10, CH63, 及 CH93 的化學結構。

Fig. 5. Glucose uptake assays for cells treated with fractions of the hexane layer and the chemical structures of CH10, CH63, and CH93. A, assays for fractions 10~13; B, assays for fractions 14~18; C, assays for fractions 19~22. All fractions were in 10μg/ml, and all were performed in triplets. Data represent mean ± SD. Dataset of each group was statistically analyzed versus that of “resistant+insulin” by two-way ANOVA. #p between groups and p of interaction both < 0.05. D, the chemical structures of CH10, CH63, and CH93.

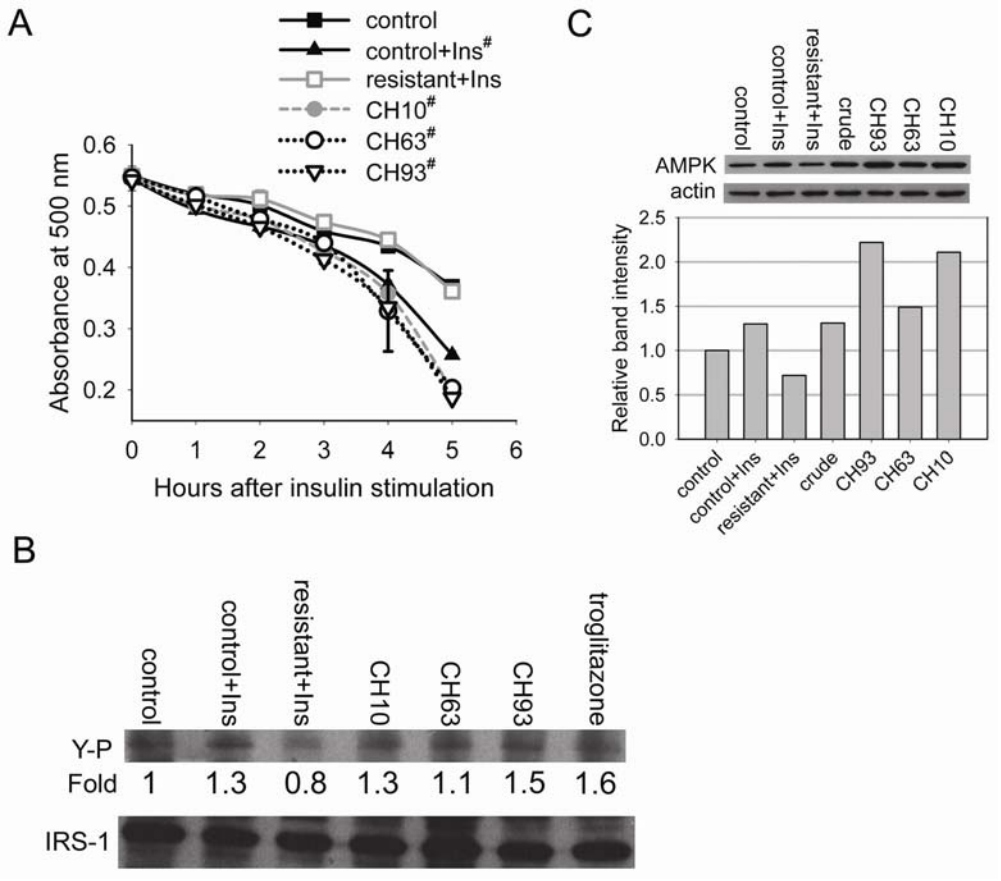

A
control
control+Ins#
resistant+Ins
CH10#
CH63#
CH93#
Absorbance at 500 nm
0.6
0.5
0.4
0.3
0.2
0
1
2
3
4
5
6
Hours after insulin stimulation
C
control
control+Ins
resistant+Ins
crude
CH93
CH63
CH10
AMPK
actin
Relative band intensity
2.5
2.0
1.5
1.0
0.5
0.0
control
control+Ins
resistant+Ins
crude
CH93
CH63
CH10
B
control
control+Ins
resistant+Ins
CH10
CH63
CH93
troglitazone
Y-P
Fold
1
1.3
0.8
1.3
1.1
1.5
1.6
IRS-1

圖 6. 分析 CH10、CH63 及 CH93 之活性。以 10μg/ml 之 CH10、CH63 或 CH93 分別處理胰島素抗性細胞。Ins, insulin. A, 分析細胞葡萄糖吸收情形，方法同圖 2。所顯示結果爲三重複實驗之 mean±SD。資料皆以 two-way ANOVA 與“resistant +insulin”分析比較。#代表 *p* between groups < 0.05 及 *p* of interaction < 0.05。B, 以 IRS-1 抗體進行免疫沉澱，再以 phosphotyrosine-specific antibody(Y-P)及 IRS-1 抗體(IRS-1) 進行西方墨點法分析。Fold 代表各蛋白質條帶相對於 control 之密度，並以 IRS-1 之條帶密度校正過；C, 以 AMPK 磷酸化狀態（代表 AMPK 之活化）之專一性抗體進行西方墨點法分析。

Fig. 6.Assay for the activities of CH10、CH63 and CH93. 10μg/ml of CH10, CH63, or CH93 was used to treat insulin-resistant cells. A, glucose uptake assays as in Figure 2. Data represent mean ± SD. Dataset of each group was statistically analyzed versus that of “resistant+insulin” by two-way ANOVA. #*p* between groups and *p* of interaction both < 0.05. B, cells were harvested at 30 minutes after the treatment. Cellular proteins were immunoprecipitated by an IRS-1-specific antibody, then analyzed by Western blotting using either a phosphotyrosine-specific antibody (panel Y-P), or the IRS-1-specific antibody (panel IRS-1). Fold represents the relative band intensities between lanes in panel Y-P that were pre-normalized by the amount of IRS-1. The band intensity of “control” was set as 1. C, Western blotting using an antibody specific for the phosphorylated form of AMPK (panel AMPK) and an antibody forβ-actin (panel actin). Cells were harvested at 30 minutes after insulin stimulation. The histogram shows the relative band intensities of phosphorylated AMPK that were normalized by the levels of actin, with the band intensity of control being set as 1.

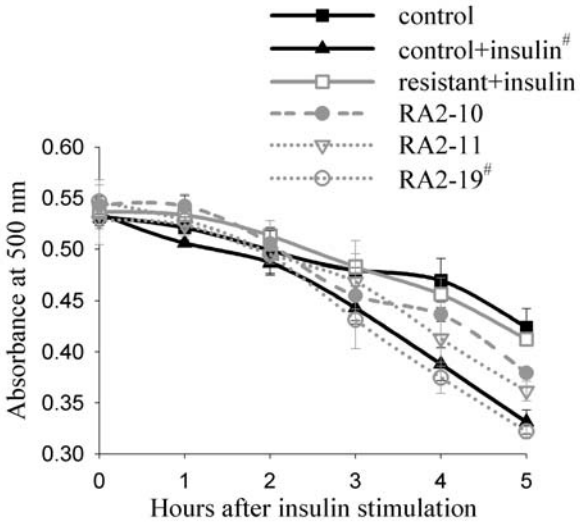


圖 7. 分析 RA2-10、RA2-11 及 RA2-19 對胰島素抗性細胞葡萄糖吸收之影響。以 10 μ g/ml 之 RA2-10、RA2-11 或 RA2-19 分別處理胰島素抗性細胞，再分析其葡萄糖吸收情形。所顯示結果爲三重複實驗之 mean±SD。資料皆以 two-way ANOVA 與"resistant +insulin"分析比較。#代表 p between groups < 0.05 及 p of interaction < 0.05。

Fig. 7. Glucose uptake assays for insulin-resistant cells treated with RA2-10、RA2-11 or RA2-19. 10μg/ml of RA2-10、RA2-11 or RA2-19 was used to treat insulin-resistant cells, and glucose uptake assays were performed as in Figure 2. Data represent mean ± SD. Dataset of each group was statistically analyzed versus that of "resistant+insulin" by two-way ANOVA. #p between groups and p of interaction both < 0.05.

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ABSTRACT

Insulin resistance plays a major role in the development of type 2 diabetes. Therefore, treatment of insulin resistance is suggested to prevent the occurrence of type 2 diabetes or improve the conditions of diabetic patients. To mimic the molecular mechanism of the development of cellular insulin resistance in vivo, we have treated mouse liver cell line FL83B with tumor necrosis factor-α(TNF-α) for the induction of insulin resistance. This established a system for the screening of hypoglycemic compounds from Momordica charantia. It was revealed that the crude extracts of stem, fruit, seed of M. charantia all contained bioactive compounds that could overcome cellular insulin resistance and improve glucose uptake of the cells. The extract from the stem of bitter gourds was further partitioned that three out of 22 fractions were with obvious hypoglycemic activities. Subsequently, pure compounds CH10, CH63 and CH93 were isolated from fractions 13 and 18. These compounds were confirmed to enhance glucose uptake and tyrosine phosphorylation of IRS-1 in insulin-resistant cells. Moreover, they were shown to activate AMP-activated protein kinase. Similar approaches were utilized to screen hypoglycemic compounds from a new strain of M. charantia , Hualien no. 2 wild bitter melon. Currently, at least one pure compound was isolated and identified to contain a similar effect. These results support that M. charantia contains components able to overcome cellular insulin resistance, suggesting a great potential of M. charantia in the development of new anti-diabetic food supplement or medicine.

Key words：type 2 diabetes, insulin resistance, *Momordica charantia,* Hualien no. 2 wild bitter melon, AMP- activated protein kinase, FL83B cells.

苦瓜蛋白調整血糖機制及其應用之研究

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摘　要

苦瓜（*Momordica charantia L.*）爲中草藥，亦是民眾日常食用之蔬果。在現代生物性療效方面，苦瓜具有治療糖尿病及其相關併發症（神經系統的損傷、白內障）的功效，也具有抗菌、抗病毒、抗腫瘤、節育、抗胃潰瘍、抗癌、治療乾癬、抗發炎和抗氧化等活性，其中以苦瓜萃取物調整血糖或應用在治療糖尿病及其併發症方面最爲顯著，但其活性成分，尤其是活性蛋白質與相關作用機轉尚未完全釐清。目前我們已完成苦瓜不同品種萃取製程蛋白萃取液調整血糖之最適化研究，證實苦瓜特定製程蛋白萃取液具有調整血糖功效，也利用區隔性分離方式，驗證調整血糖之最佳分子量區間苦瓜蛋白及可能之活性物質。進一步應用蛋白質體學技術分析具調整血糖功能苦瓜萃取液蛋白質之組成成分，並探究苦瓜萃取液調整血糖機轉，以完成化學組成固定、生物反應明確、無生物安全性顧慮之苦瓜調整血糖保健產品之開發。由本研究的執行，預期可確定苦瓜調整血糖的功效，進一步分析其有效機轉及組成份，未來應可發展爲治療或輔助糖尿病治療的中草藥保健食品，甚至是導引糖尿病之新藥開發。

關鍵詞：苦瓜，調整血糖，保健產品，蛋白質體學

前言

開發具調整血糖活性之中醫藥新藥的系列產品，建立跨國性具產業應用性之智慧財產，是輔助生技產業開發之重要項目。調整血糖製劑在市場規模與範圍之預估方面，根據 WHO 的相關統計顯示，糖尿病病患的人口總數一直呈現驚人的成長，從 1985 年的 3 千萬人、1995 年的 1 億 3 千 5 百萬人，到今日全球糖尿患者總數已超過 1 億 7 千 7 百萬人，而 WHO 更進一步指出，到 2030 年時，全球罹患糖尿病的總人口將爆破 3 億 3 千 6 百萬人。此外，糖尿病相關疾病所造成的死亡人數，已佔全球每年死亡總人數的 5%左右，而且 WHO 警告若各國再不積極的預防及治療糖尿病及其相關疾病，未來 10 年可預期因糖尿病相關疾病所造成死亡的總人數將再激增一倍（http://www.who.int/diabetes/en/）。在與糖尿病直接相關的醫療費用統計方面，糖尿病的每年醫療費用幾乎佔各國健康衛生相關預算總額的 2.5%到 15%。反觀國內健保用在糖尿病的醫療費用方面，90 年時約 271 億元，91 年健保糖尿病人醫療費用約 317 億元，年增率約 17%，所以糖尿病在國內亦是影響健保支出的重要因素。如果開發具調整血糖活性之中醫藥新藥的系列產品，一方面可提高台灣中醫之能見度，另一方面可以降低健保的負擔。而且台灣目前市售調整血糖的保健產品，多爲未經醫師處方的中草藥相關產品、民間偏方或傳銷商品，其產品多未經研究證實其療效，在製程上是否經有 GMP 認可亦不清楚，民眾服用後其效果堪慮，若有進一步問題發生，又多增健保醫療費用等社會成本的支出。所以如能開發出療效明確的具調整血糖活性之中醫藥新藥的序列產品，應能降低相關事件的發生，亦能增進國民的健康。

糖尿病是重要的慢性病之一，主要可分爲胰島素依賴型與非胰島素依賴型糖尿病。胰島素依賴型的糖尿病病人，其胰臟無法生產胰島素，又稱做第一型糖尿病。非胰島素依賴型糖尿病病人則是胰島素分泌不足或對胰島素產生耐受性所導致，又稱做第二型糖尿病。無論第一型糖尿病或第二型糖尿病，人體皆會有高血糖的病徵，或是長時間處於高血糖的體液環境中，體內滲透壓過高，容易造成人體神經系統的持續傷害，以及讓整個血液循環系統壓力變大，導致日後神經與血管的病變產生。然而，對於糖尿病致病機轉的研究中，也發現有許多訊息傳遞路徑與葡萄糖的代謝相關，例如，葡萄糖的代謝、特定器官對葡萄糖的攝取路徑等。近年，除了從 insulin receptor 或是 glucose transporters 的研究方向去研究糖尿病的治療外，目

前認爲轉錄因子 peroxisome proliferator-activated receptors（PPAR）是治療的新標的物（Rosenson, 2007），此外已有文獻報告指出一些自然界作物在治療或改善糖尿病的主題中慢慢嶄露頭角（Iwashima et al., 2001; Storling et al., 2005; Zhao et al., 2005a），這些觀念也逐漸被用來作爲治療糖尿病體內試驗的重要發展方向。因此由 insulin receptor，到 PPAR 相關基因及 glucose transporters，已成爲糖尿病及其藥物研發一主要路徑。

由上述觀點轉換成研究平台，在體外試驗部份，對於胰島素耐受性所導致脂肪傷害的研究中，以細胞株 murine 3T3-L1 cells（Karalee et al., 2001）與 GLUT4（Maddux et al., 2001）做爲 glucose uptake 機轉探究的基礎。脂肪組織過多的形成堆積，與肥胖、第二型糖尿病有著密切關係，且會進一步導致脂肪中毒產生造成細胞傷害，使細胞內脂肪細胞對胰島素產生耐受性（Lelliott and Vidal-Puig, 2004）。若細胞對胰島素產生耐受性，則會於脂肪細胞以及平滑肌中形成高血糖的狀態；然而，由不同部位產生之脂肪細胞被傷害後，也會產生不同的生理或病理的機制反應。

在體內動物實驗部份，嚙齒類動物是最佳的實驗動物種類，也有學者將老鼠的基因做修飾後，成爲 knockout 的小鼠或是基因轉殖鼠，藉此進一步研究對糖尿病的影響機轉（Rees and Alcolado, 2005; Masiello, 2006）。糖尿病的誘因可以藉由藥物的不當使用、意外傷害或是基因遺傳層面上，導致不同動物糖尿病的發生。在 1996 到 2006 年之間，許多民族重要天然物治療糖尿病的研究文獻投稿於 Journal of Ethnopharmacology 中，多利用此實驗模式，其中，Streptozotocin（STZ）以及 alloxan 誘發糖尿病動物模式居多，各有 69%與 31%的出現率，其會誘發高血糖產生，但與人糖尿病病程差異度較大。至於 knockout 的小鼠或是基因轉殖鼠中，以 ob/ob mouse 最廣爲被使用。Ob/Ob mouse，其血糖值能夠維持在 euglycemia（80-110 mg/dl ）以及持續補償對胰臟 ß-cell 反應的狀態，保持在高胰島素血症的情況下與胰島素耐受性相抗衡著。Ob/Ob mouse 模式中，胰島素的耐受性大多是由於過度肥胖、高血脂以及高血糖所引起，上述這些症狀也同時能夠在第二型糖尿病病人中被發現。

傳統對於糖尿病的治療，現代藥物的使用率較中草藥來的廣泛，也因爲不同的現代藥物在使用上有藥物副作用的堪慮，Li et al.（2004）多次評論在臨床上常用來治療糖尿病的傳統中草藥處方，或者是治療由糖尿病所引起的其他併發症等，發現中草藥在對於糖尿病及其相關的預防治療上有

加重的趨勢。因此，我們將這些在文獻中已有使用的中草藥重新統整，其中也有已找到其生物活性成分的天然化合物（Chen, 1987; Wu and Li, 1992; Gu and Jiang, 1997; Yin and Chen, 2000），這些具備活性成分的構造包含 polysaccharides、terpenoids、flavonoids、sterols、alkaloids 及其他等。上述已發表的天然化合物中，由於 polypeptides 類成分的研究甚少，及由 insulin receptor，到 PPAR 相關基因及 glucose transporters 之糖尿病藥物研發路徑判讀，polypeptides 類成分較有可能在上游產生效果。因此研究調節血糖相關 polypeptides，應較具有新穎性與可專利性，未來進一步希望發展成新藥開發的新標的，以及用在臨床上治療糖尿病的新契機。

苦瓜是葫蘆科 *Momordica charantia* L.的果實，廣泛分布於亞洲、亞馬遜河流域、東非等地，因此許多民族經常把苦瓜列入傳統藥物之一，用於治療及預防一些慢性疾病，如糖尿病、經痛、痛風、濕疹、節育、墮胎、腎結石、肺炎、乾癬等方面。在台灣，苦瓜同時也是一般民眾經常食用的蔬果之一，而在中醫藥重要的典籍中亦有記載，如本草綱目中認爲苦瓜有“苦寒、無毒、除邪熱、解勞乏、清心明目”等功效。在現代生物性療效方面，苦瓜也有近百篇的相關文獻，其中主要有針對幾個方向，如治療糖尿病及其相關的併發症（神經系統的損傷、白內障）、抗菌、抗病毒（HIV、HSV）、抗腫瘤、節育、抗胃潰瘍、抗癌、治療乾癬、抗發炎和抗氧化等活性（Grover and Yadav, 2004），而其中以苦瓜萃取物調整血糖或應用在糖尿病及其併發症最爲顯著，但這些研究多偏重在動物實驗，少有苦瓜調整血糖之相關機制及其功能性成分之研究。

目前有關苦瓜在糖尿病方面的研究有降低正常動物血糖（Bailey et al., 1985; Day et al., 1990; Shibib et al., 1993; Ali et al., 1993; Cakici et al., 1994; Sarkar et al., 1996; Jayasooriya et al., 2000）、抗 alloxan 及 streptozotocin 所誘發的高血糖（Akhtar, 1982; Karunanayake et al., 1984; Singh et al., 1989; Pari et al., 2001; Rathi et al., 2002a; Kar et al., 2003; Kedar and Chakrabarti, 1982; Bailey et al., 1985;Day et al., 1990; Karunanayake et al., 1990; Higashino et al., 1992; Shibib et al., 1993; Sarkar et al., 1996; Ahmed et al., 1998; Ahmed et al., 2001; Sitasawad et al., 2000; Grover et al., 2002; Rathi et al., 2002）、增加胰臟功能而達到降血糖的作用（Day et al., 1990; Sarkar et al., 1996）、增加 beta cells 的數目（Ahmed et al., 1998）、促進胰島素分泌及胰島素樣作用（Welihinda et al.,1986; Higashino et al., 1992）、抑制糖尿病大鼠肝中

hexokinase, glucokinase, phosphofructokinase 及受質 glucose-6-phosphate 的活性（Rathi et al., 2002）等。而根據相關研究指出，苦瓜的化學成分有皂苷、生物鹼、三萜、固醇及蛋白質等成分，其中固醇類皂苷化合物，如 charantins，及一些蛋白質成分被認爲具有降血糖的活性（Raman and Lau, 1996）。在許多種類的植物中，皂苷類成分的降血糖活性已有許多相關研究，甚至已有文獻研究該類成分結構與降血糖活性之關係（Matsuda et al., 1998）。但是相對於其他植物成分的研究，苦瓜中已分離出具降血糖活性的蛋白質成分，在糖尿病動物模式的研究相對較少，且也缺乏進一步的分子機轉的研究，因此我們希望鎖定小分子量的蛋白質，設立萃取小分子量蛋白質的萃取製程，以進一步針對苦瓜機能性蛋白質進行深入的研究。

機能性食品必須具備含有已被闡明化學結構的功能因子且功能因子在食品中穩定存在等要件，而苦瓜中負責調整血糖的功能因子尚需進一步之研究，因此，實驗室擬由已建立之調整血糖分析系統，對台灣省產之各苦瓜品種進行評估，並以胰島素信息傳導路徑分析其調節血糖之相關機轉。進一步再經由不同萃取方法搭配分析化學及蛋白質體學技術等方法，搜尋苦瓜中功能性成分及其對胰島素信息傳導的影響。經由本研究的執行，預期可發現苦瓜中的功能性成分，未來應可進一步發展爲治療或輔助糖尿病治療的中草藥保健食品。

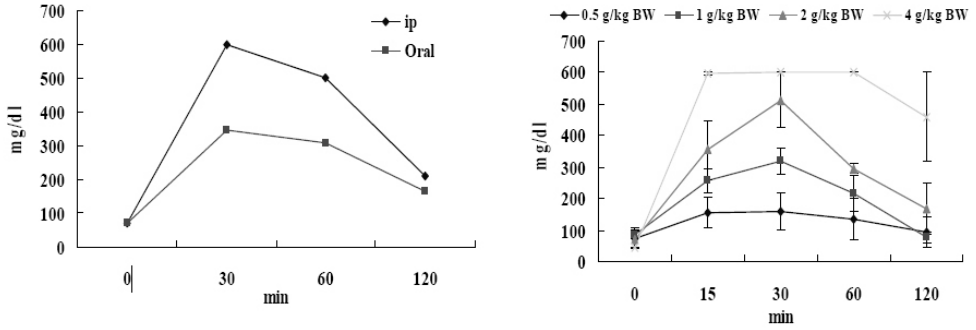
內容

苦瓜爲中草藥，本草綱目中認爲苦瓜有“苦寒、無毒、除邪熱、解勞乏、清心明目”等功效，而其中以苦瓜萃取物調整血糖或應用在治療糖尿病及其併發症方面最爲顯著，但這些研究多偏重在動物實驗，少有苦瓜調整血糖之相關機制及其功能性成分之研究。一般而言，機能性食品必須具備含有已被闡明化學結構的功能因子且功能因子在食品中穩定存在等要件，而苦瓜中負責調整血糖的功能因子尚需進一步之研究。因此，實驗室已建立之調整血糖的動物模式，已確定苦瓜特定製程蛋白萃取液具調整血糖功效，進一步也利用區隔性分離方式驗證調整血糖之最佳分子量區間苦瓜蛋白。

目前已建立超音波震盪、透析、分子量及等電點分離等不同苦瓜特定品種萃取製程，已確定特定品種苦瓜之萃取液在動物試驗中具調整血糖功效。因從上述 Khanna 等人的研究中發現苦瓜調整血糖的功能性蛋白主要

爲小分子量的蛋白質，爲了快速分析出苦瓜中功能性蛋白質之類型，我們分別以熱水、水、甲醇、乙醇、酸性乙醇等溶媒萃取不同極性的蛋白質成分，並將各種苦瓜的不同部位直接均質後萃取蛋白質成分，再以 SDS-PAGE 分析其小分子量蛋白質所佔之比例和比較各品種苦瓜蛋白質之差異，以找出萃取苦瓜小分子量蛋白質最有效的方法。進一步搭配凝膠層析（gel filtration） 等方法，製備苦瓜可能調整血糖之區隔性成分。本項研究中主要以活性分析平台及動物模式交叉實驗，引導苦瓜活性蛋白的萃取與純化策略。

調整血糖動物模式之建立根據 Day 等人的方法，建立正常動物降血糖模式。動物模式是以 8 週齡、體重 20±1 公克的雌性 BALB/c 小鼠爲實驗動物，在禁食 18 小時後，分別以口服或腹腔注射方式給予高濃度的葡萄糖溶液（2g/kg 體重）。經實驗證實葡萄糖溶液以腹腔注射的方式給予小鼠，可以使小鼠維持穩定的高血糖狀態（左圖）。而以腹腔注射葡萄糖溶液（0.5~4 g/kg 體重）進行劑量反應的測試，發現血糖濃度會隨著葡萄糖注射濃度的上升而增加，在腹腔注射 30 分鐘後，血糖濃度會達到高峰，而 2 小時後，血糖濃度會下降成背景值（右圖）。因此我們利用每公斤體重 4 公克的葡萄糖濃度、腹腔注射的條件，進行後續苦瓜萃取液調整血糖的實驗。



目前已確定至少一種苦瓜特定製程蛋白萃取液（MCSE）調整血糖的效果。將不同濃度的苦瓜特定製程蛋白萃取液以口服方式的方式投與小白鼠，再注射葡萄糖溶液（4g/kg 體重）後，檢測其血糖的變化。結果顯示，血糖濃度隨著苦瓜特定製程蛋白萃取液濃度的增加而下降，而於 2 小時後，血糖濃度會下降成背景值，證明苦瓜特定製程蛋白萃取液確實具有調

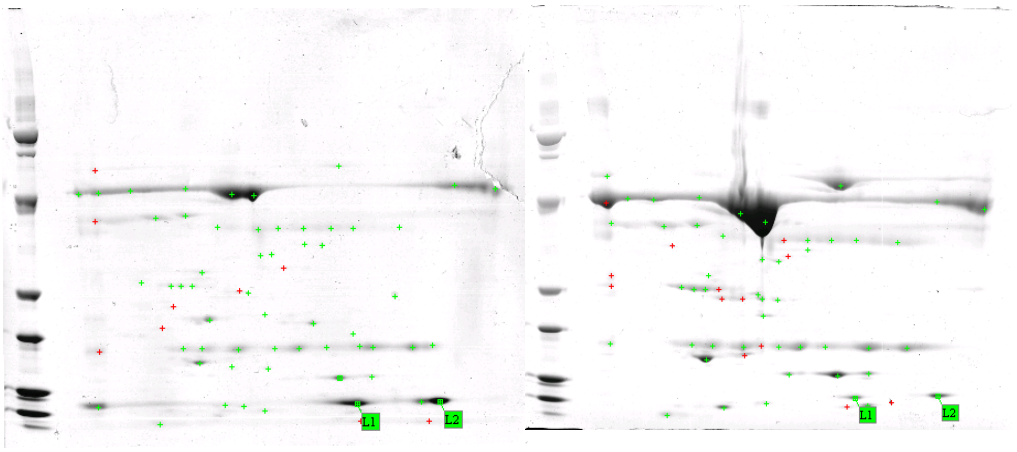
整血糖的功能。

苦瓜特定製程蛋白萃取液之療效進一步以糖尿病動物模式分析其療效，糖尿病有許多動物模式，因本研究希望以第二型糖尿病爲主要之標的，因此經由本研究團隊過去之經驗，選擇果糖誘導的胰島素耐受型 Sprague-Dawley（SD）大鼠糖尿病模式，進行苦瓜蛋白質萃取液在調整血糖方面療效的活性評估（Chen et al., 2001）。依照本團隊過去的研究顯示，高果糖飲食組的胰島素及游離脂肪酸含量約爲正常組的兩倍，而在三酸甘油脂方面則約爲 2.5 倍，在陽性對照組方面則降回背景值左右。故本實驗以 150-200g SD 大鼠，分別給予正常與高果糖的飲食連續 6 週。陽性對照組與苦瓜組則分別在高果糖飲食中同時給予 troglitazone 和苦瓜蛋白萃取液或其功能性成分進行治療，最後再測定其血壓、胰島素、游離脂肪酸、三酸甘油脂等相關生化數值之改變。

除了 SD 大鼠糖尿病模式外，另有其它胰島素非依賴型糖尿病動物模式，以雙重驗證苦瓜蛋白質萃取液調整血糖的活性（Luo et al., 1998; Apweiler and Freund, 1993; Chen and Wang, 2005; Rees and Alcolado, 2005）。動物模式的建立方法如下：以 3 週週齡的 C57BL/6J 小鼠，分別給予正常與高脂肪（35.5%脂肪）的飼料 3 週後，再以 STZ 100mg/kg 誘發糖尿病，對照組則注射等體積之溶媒（0.05 M citric acid, pH4.5），持續給予相同之飲食。在 STZ 注射後的適當時間給予不同的苦瓜蛋白萃取液治療及抽血檢測其血糖之變化，陽性對照組則投予 tolbutamide（200 mg/kg）進行治療。STZ 注射後的第 31 天將小鼠犧牲採集其血液與相關組織，進行血糖、組織切片與糖尿病控制相關機轉之檢測。以 6~7 週的 Obese（fa/fa）Zucker 大鼠正常飼養 12 週，待其自行產生胰島素耐受性及高血糖等糖尿病症狀後，再給與藥物治療並觀察相關生化數值的變化。

本實驗以上述動物模式爲基礎，將測試不同苦瓜萃取液在不同動物模式交叉實驗，以引導較高療效，較低成本之調整血糖保健產品之開發。應用蛋白質體學技術，搜尋具調整血糖功能苦瓜萃取液中蛋白質組成成分，利用苦瓜可能調整血糖之區隔性成分爲基礎，以 2D-PAGE-MS 進行其組成蛋白質成分之鑑定與分析。簡述如下：將製備好的苦瓜各部位樣品，先以二維凝膠電泳分析，再以影像成像系統擷取並分析其結果。之後，切下欲檢測蛋白質的點並消化後，再以質譜儀進行序列分析，最後將胺基酸序列利用蛋白質資料庫進行搜尋比對。蛋白質序列比對資料經生物資訊學工具

分析，歸類其蛋白質家族，推測可能功能後，進一步分析基因啓動子上轉錄因子結合位的種類，研究其生合成的調控機制。下圖爲本研究團隊利用已建立之蛋白質體相關技術平台，進行兩個不同部位的蛋白質二維凝膠電泳分析之初步成果，相同點以綠色表示，相異點以紅色表示。



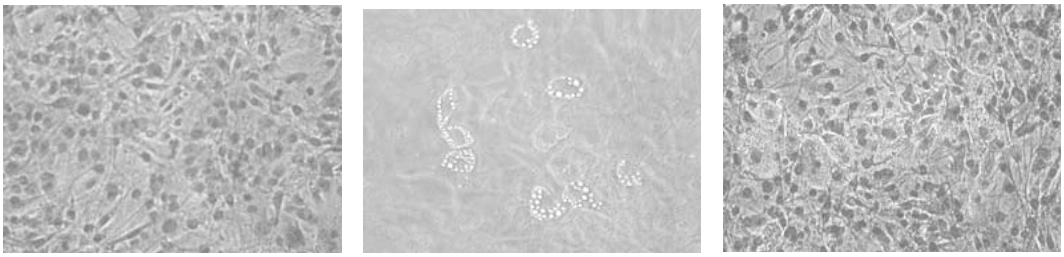
在此蛋白質體相關技術平台中，兩個不同部位的蛋白質二維凝膠電泳分析選擇最具有意義的點進行液相層析串連質譜儀。液相層析串連質譜儀水解特定胜肽所形成之圖譜，經資料庫比對後可分析出胺基酸序列。最後將相關序列經 NCBI 蛋白質資料庫進行比對分析，分析其胺基酸序列。二維凝膠電泳分析選擇最具有意義的點，至少需分析 5 段胺基酸序列，其中至少須有 3 段胺基酸序列與 NCBI 蛋白質資料庫比對分析具高度相似性，方能判讀其蛋白質之種類。

臨床上治療糖尿病的藥物，主要是以 biguanides、sulphonylureas 、thiazolidinediones、D-phenylalanine 衍生物等藥物爲主，而這些藥物的共同點都是藉由影響胰島素分泌或改變其訊息傳導路徑來發揮其療效。因此，可先將具有胰島素受體的細胞株 3T3-L1 誘發分化成脂肪細胞後，處理不同的苦瓜萃取物或其成分，再投與放射線標定的葡萄糖後，觀察各種處理組的細胞是否會將葡萄糖攝入細胞內，以分析不同的苦瓜萃取物或其成分是否具有調整血糖的功能。

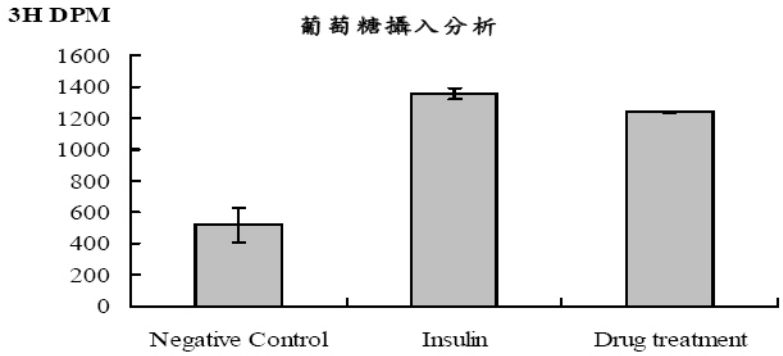
下圖爲本研究團隊誘發細胞分化的初步結果，分別是以顯微鏡在固定放大倍率（200X）下觀察 3T3-L1 在誘發分化前後的細胞形態變化。左圖爲在 Oil Red 染色下，觀察 3T3-L1 原來的細胞形態，3T3-L1 呈現出原本

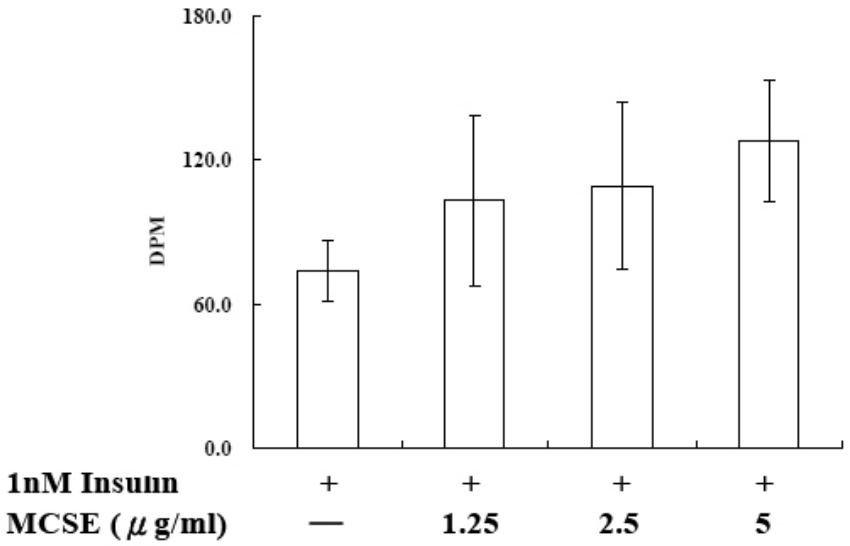
的纖維母細胞的形態特徵。中間圖則爲 3T3-L1 在誘發分化後所形成的脂肪細胞，從圖中可觀察到細胞內具有明亮的油室，此即是脂肪細胞的特徵之一。右圖是中間圖的細胞再經由 Oil Red 染色後進行觀察，從圖中可以更清楚的看到被染成紅色的油室，證明 3T3-L1 已經確實被誘導分化成脂肪細胞。

Preadipocytes（Oil Red） adipocytes adipocytes（Oil Red）



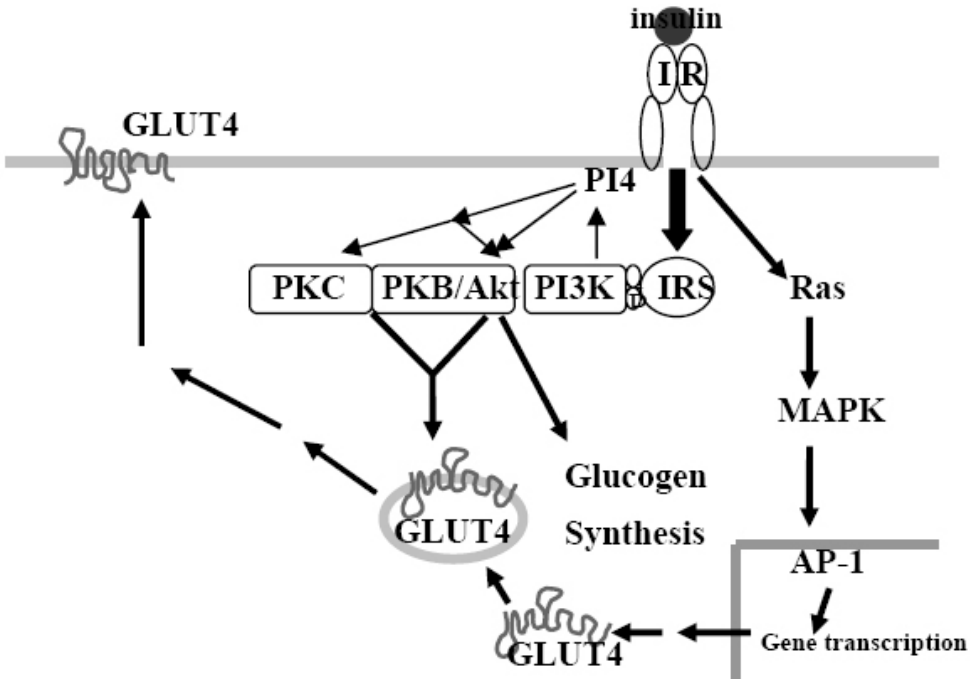
下圖爲 3T3-L1 分化成的脂肪細胞經胰島素及藥物等處裡後，其對氚（3H）標定葡萄糖的不同攝取能力。陰性對照組只有誘導分化未做任何其他處理，以胰島素刺激葡萄糖的攝取爲陽性控制組，最後給予藥物看藥物是否能刺激其葡萄糖的攝取。由下圖結果顯示藥物可以成功的促進脂肪細胞葡萄糖的攝取。





上述苦瓜特定製程蛋白萃取液（MCSE）調整血糖的效果可能與促進3T3-L1脂肪細胞攝取葡萄糖有關。3T3-L1分化成的脂肪細胞後，再經胰島素或不同濃度的MCSE處理，測定對氚（3H）標定葡萄糖的攝取能力。結果顯示隨著MCSE濃度的上升，細胞攝取葡萄糖的能力也增加，證明MCSE可以藉由促進細胞攝取葡萄糖，而降低血糖濃度。

3T3-L1細胞經胰島素刺激後，會經由下圖兩條主要路徑調節葡萄糖轉運分子（GLUT4） 在細胞膜上的量，以促進細胞膜外的葡萄糖攝入細胞內。第一條主要路徑是經由胰島素刺激後活化insulin receptor-β subunit(IR)，之後藉由將insulin receptor substrate磷酸化後，活化phosphatidylinositol 3-kinase（PI3K），再經由相關路徑，活化下游的protein kinase C（PKC）和Akt，最後促進GLUT4轉運與細胞內的糖質新生。另一條路徑是經胰島素刺激活化Ras 後，活化MAPK相關路徑之轉錄因子（包括PPARγ、AP-1），增加GLUT4的產生，以達到降低血糖的反應。因此本研究擬以Western blot分析相關訊息傳導、以electrophoretic mobility shift assay 探討對轉錄因子的影響及以免疫螢光分析法測定細胞表面GLUT4的 translocation，分析苦瓜機能性成份調整血糖之可能機轉。本項研究與本校附醫新陳代謝科陳清助醫師合作執行。



化學組成固定、生物反應明確、及無安全性顧慮之苦瓜調整血糖保健產品之開發，主要以活性分析平台及動物模式交叉實驗，引導苦瓜最大活性物質的萃取策略的製定。未來如進一步確認某一種蛋白質爲苦瓜中之可能相關調整血糖因子，進一步可設計爲大量富集活性蛋白及固定蛋白質組成的苦瓜萃取製程。目前已確定在苦瓜在在特定分子量區間具較佳之血糖調整效應。

苦瓜調節血糖保健食品的產品設計可與產業界協同進行。由本研究篩選出具調整血糖之功能性蛋白後，配合前述的苦瓜特定製程蛋白萃取液分析，找出哪一品種苦瓜的特定部位含量最高，再以不同的方法進行活性蛋白質的萃取及純化，進一步以本研究的活性平台與動物實驗進行驗證，製訂出合理的各種活性蛋白含量範圍。之後再採用cGMP之製程將市售苦瓜，進行萃取與部分純化後，製造成苦瓜調整血糖保健食品之原型產品，在原型產品療效確認後，同時確立並修正其製程，以加速相關保健產品的開發時程。

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ABSTRACT

Bitter gourd（*Momordica charantia* L.） is a commonly known vegetable and medicinal herb. Severalscientific papers indicated that bitter gourd extracts are used for the treatment of diabetes and its complications, such as nephropathy, cataract and insulin resistance, by regulating the levels of blood glucose. However, the active components of bitter gourd responsible for hypoglycemic activities remained to be identified. In the first-year project, we have demonstrated that the protein extracts of bitter gourd exhibited the hypoglycemic activities by glucose tolerance test in mice. By molecular weight cutting assay, we identified that specific protein components were responsible for the regulation of blood glucose. In the second- year and third-year project, we will compare the effects of various species and parts of bitter gourd on the regulation of blood glucose. The protein components of bitter gourd will be identified by proteomic approach and the hypoglycemic mechanism of protein components will be demonstrated by molecular biology approach. The chemical defined, bioactivity defined, and biosafe bitter gourd health food for the regulation of blood glucose will be further developed. The better understanding of the functional proteins could provide the molecular evidences for the hypoglycemic activity of bitter gourd and further develop as the health food for the improvement of diabetes.

Key words：bitter gourd, hypoglycemic activity, health food, proteomics

農業生物技術國家型計畫

抗代謝症候群苦瓜保健食品研發-子計畫 1

苦瓜活化PPAR成份之萃取與化學鑑定及其供為調節胰島素敏感性與血脂保健食品之研發

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1

Table 1. Abnormalities associated with the metabolic syndrome
(modified from [Reaven, 2002])

The core Cluster

- Central Obesity
- Dyslipidemia
  - hypertriglyceridemia
  - -low HDL cholesterol
  - -small, dense LDL particles
- Glucose intolerance
  - -impaired fasting glucose
  - -impaired glucose tolerance
  - -type 2 diabetes
- Insulin resistance
- Hypertention

Prevalence: ~1/4 in USA, 14% in Taiwan

2

Definition of metabolic syndrome
World Health Organization

◆ **The patient must have 1 of the following:**

- Diabetes mellitus
- Impaired glucose tolerance
- Insulin resistance

◆ **Plus any 2 of the following:**

- Central obesity
- Dyslipidemia
- Hypertension

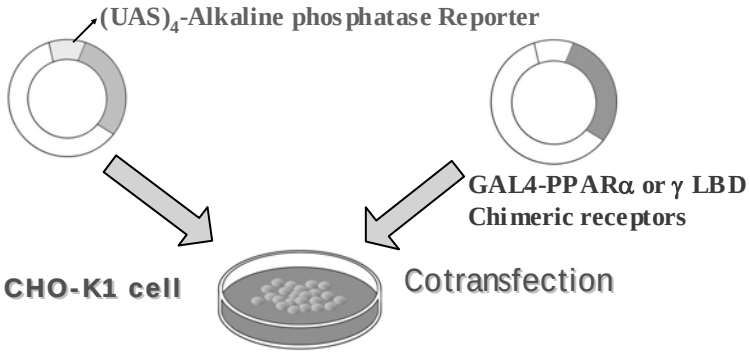
3

AmJ Clin Nutr 2006;83:1237– 47.

PPARs :
Peroxisome **P**roliferator **A**ctivated **R**eceptor

- **Ligand-activated transcription factors**
- **Member of**
  Steroid hormone nuclear receptor superfamily
- **PPARα為fibrates類降血脂藥物之作用分子標的**
- **PPARγ為Thiazolidinedione類抗糖尿病藥物之作用分子標的**
- **PPAR 活化物促進cholesterol reverse transport**
- **PPAR活化物抑制巨噬細胞主導之發炎反應與動脈粥狀硬**

Transactivation Assay for Nuclear Receptors



↓ 5 hours

Treat sample extract, or positive control (WY-14643 or BRL-49653)

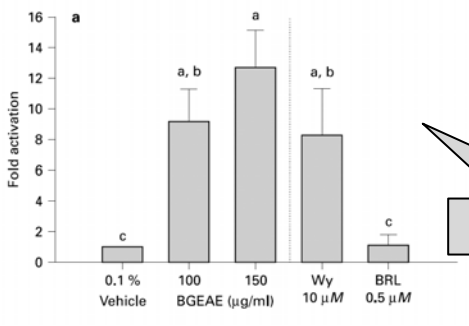
↓ 48 hours

Collect medium and ALP activity assay (ELISA)

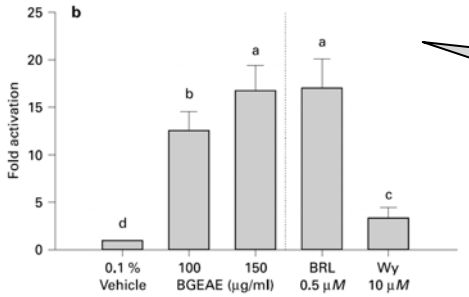
5

EA extract of bitter gourd





PPARα activation



PPARγ activation

⇩

It is a dual agonist

6

J Biomed Sci 10: 782-791

Animal Study :
Bitter Gourd *(momordica charantia)* and Metabolic syndrome

Improvement study 改善

Experimental I: High **fat diet - C57BL/6J mice**

Prevention study 預防

Experimental II: High fat diet- Wistar Rats
Normal diet- **C57BL/6J mice**

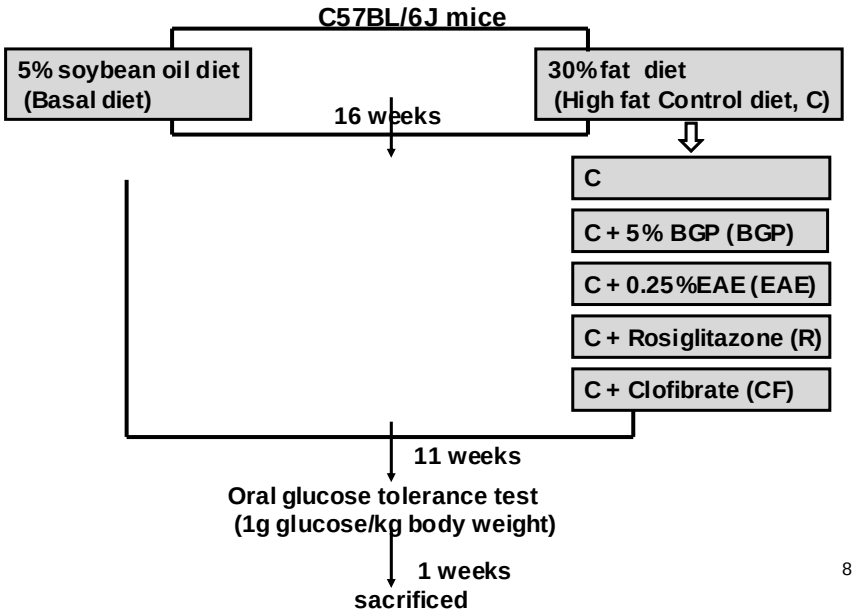
Dose-Response study 劑量

Experimental III: High fat diet-Wistar Rats
Normal diet- **C57BL/6J mice**

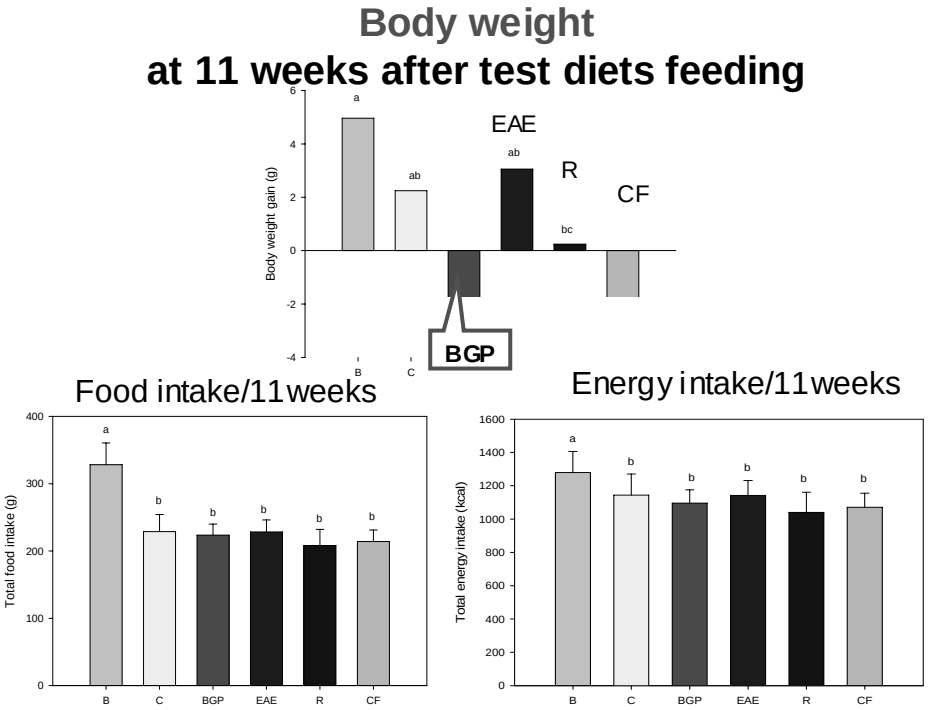
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Exp. I: Improvement study

High fat diet induced metobolic syndrome



8


Body weight
at 11 weeks after test diets feeding
Body weight gain (g)
EAE
R
CF
BGP
Food intake/11weeks
Total food intake (g)
Energy intake/11weeks
Total energy intake (kcal)
B
C
BGP
EAE
R
CF


Plasma glucose
at 4 and 11 weeks after test diets feeding
mg/dL
Weeks
B
C
BGP
EAE
R
CF

(二) OGTT

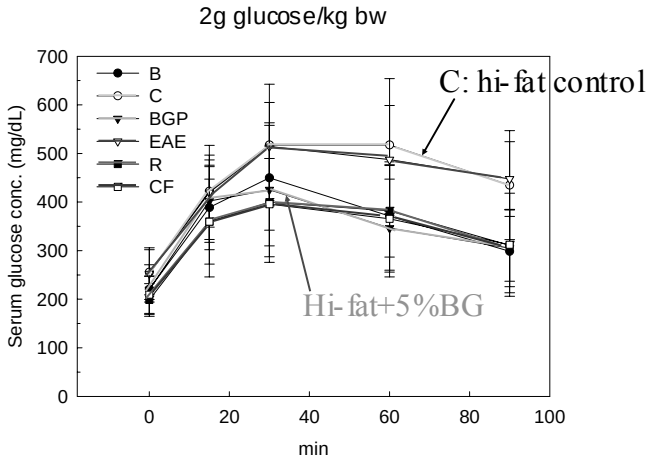
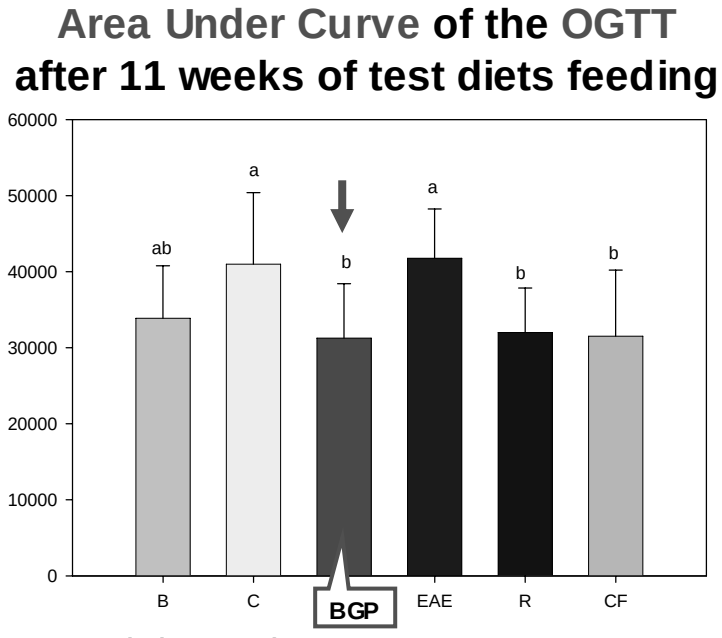


Fig. 7 B6 餵食實驗飼料九週後之 OGTT 結果

六組實驗動物皆於實驗進行三十分鐘後血糖達最高值

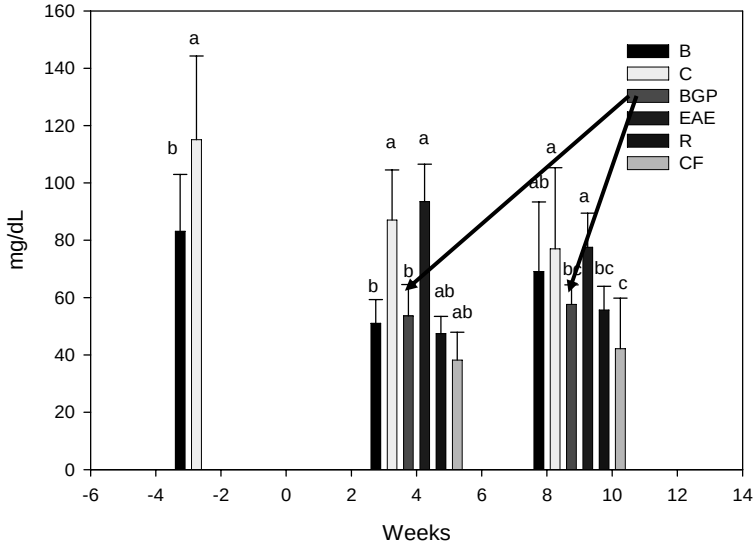
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OGTT: Oral Glucose Tolerance Test

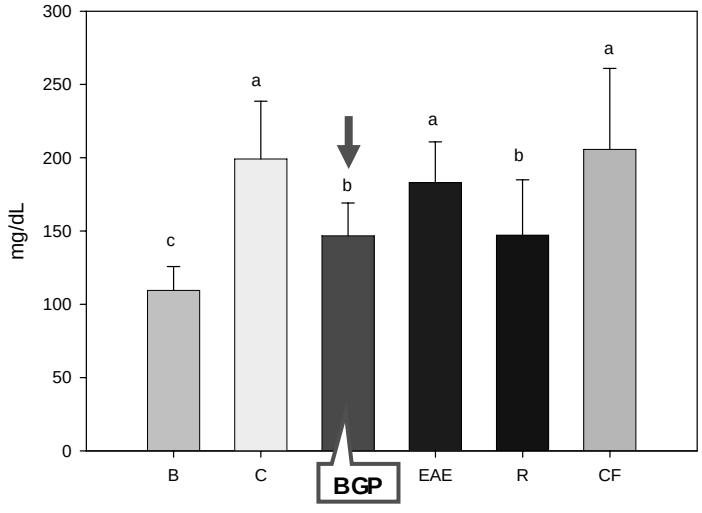
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Plasma TG concentrations at 4 and 11 weeks of test diets feeding



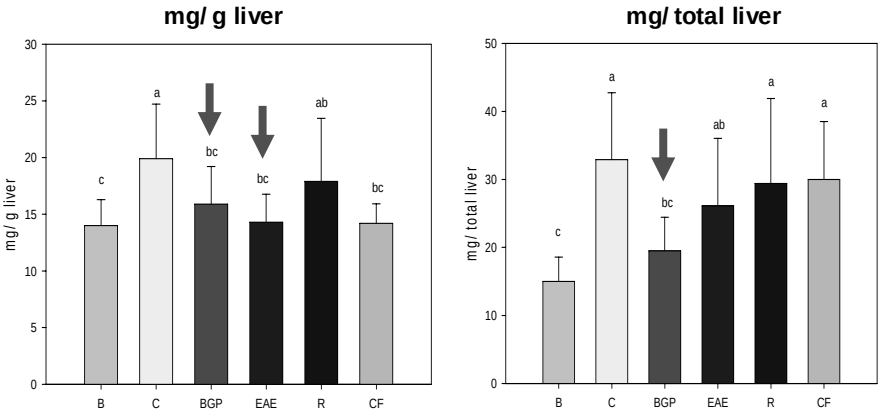
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Plasma cholesterol concentrations after 11 weeks of test diets feeding



14

Liver TG
after 11 weeks of test diets feeding



15

Summary

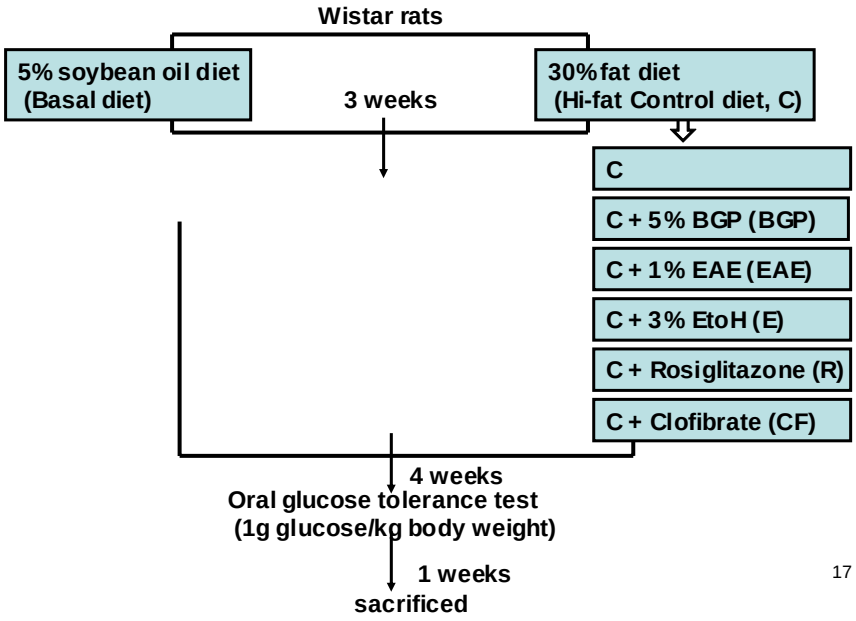
- **5% bitter gourd significantly improved**
  1. Body weight gain
  2. Hyperglycemia
  3. Glucose intolerance
  4. Hypertriglyceridemia
  5. Hypercholesterolemia
  6. Liver TG accumulation

- **0.25% EAE of bitter gourd**
  ⇨ameliorated the liver TG accumulation

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Experimental II: Prevention study

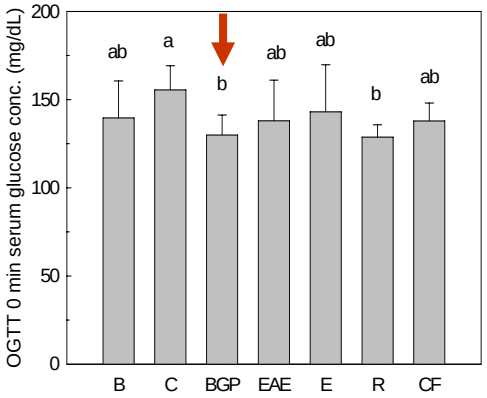
A: High fat diet induced metabolic syndrome



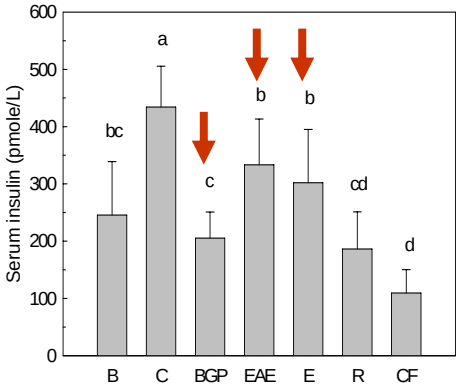
17

Plasma glucose and insulin at 4 weeks of test diets feeding

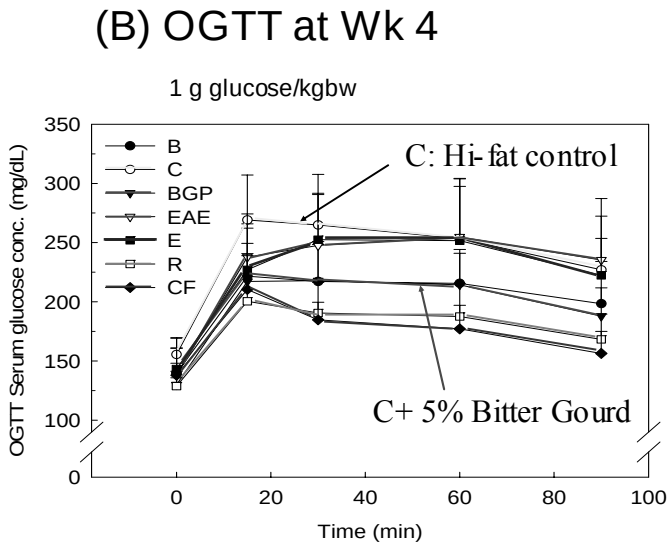
glucose



insulin



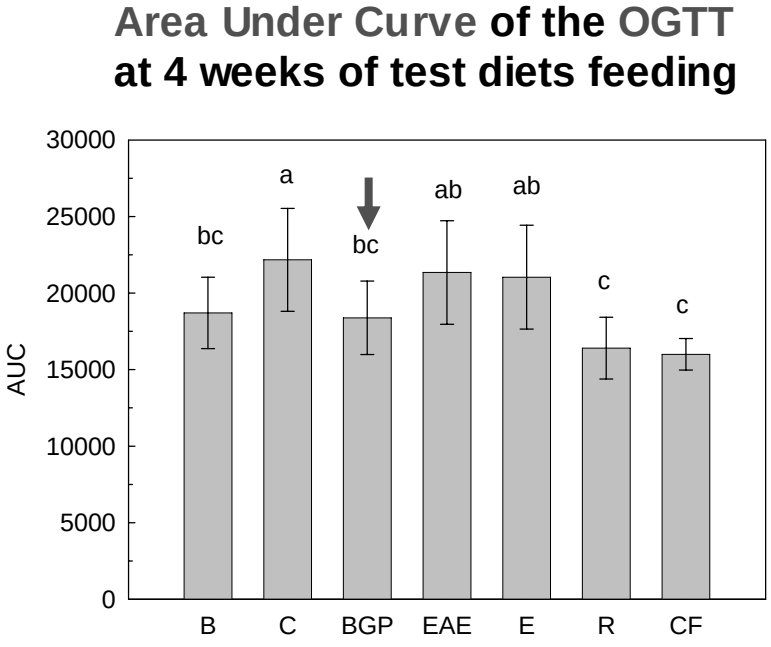
18



B, C, R, CF 組於實驗進行 15分鐘後血糖達最高值

BGP, E 組於實驗進行 30分鐘後血糖達最高值

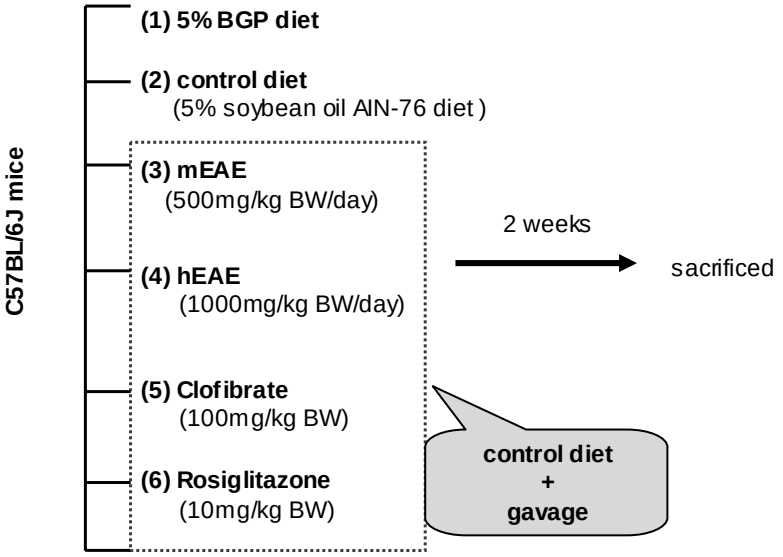
EAE 組於實驗進行 60分鐘後血糖達最高值 19



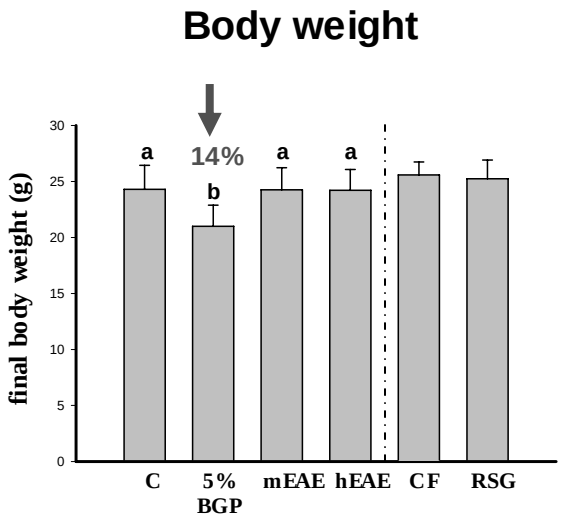
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Experimental II: prevention study

B: Freeze-Dried Wild Bitter Gourd diet effects on C57BL/6J mice



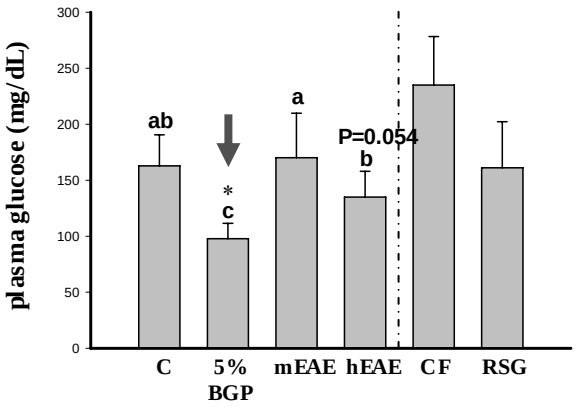
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(Food and energy intake have no significant difference)

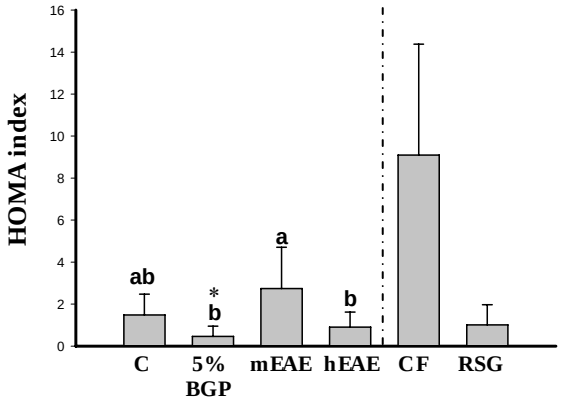
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Plasma glucose concentrations



23

HOMA index



HOMA (homeostasis model assessment) index
=[fasting plasma glucose(mmol/L) x fasting plasma insulin (μM/mL)/22.5] 24

Summary

◎ **High fat diet induced obesity (Wistar rats)**

◆ **5% bitter gourd significantly prevented**

1. **Hyperglycemia**
2. **Hyperinsulinemia**
3. **Glucose intolerance**

◆ **1% EAE of bitter gourd significantly prevented**

Hyperinsulinemia

◎ **C57BL/6J mice**

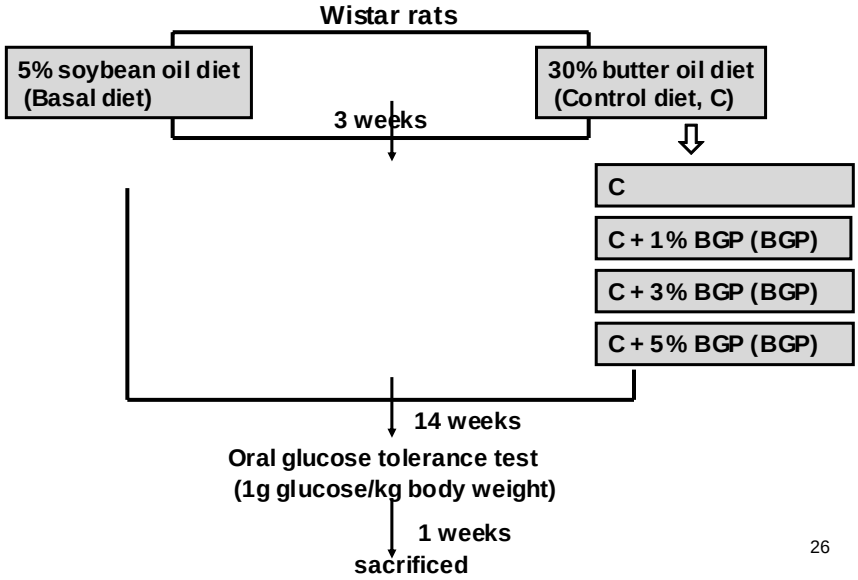
◆ **5% bitter gourd significantly prevented**

1. **Body weight gain**
2. **Hyperglycemia**

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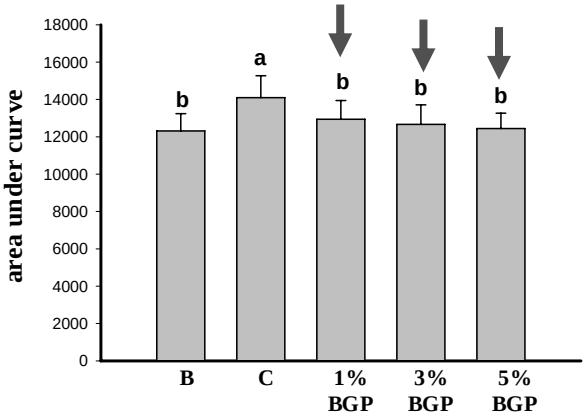
Experimental III: dose-response study

A: High fat diet induced metabolic syndrome



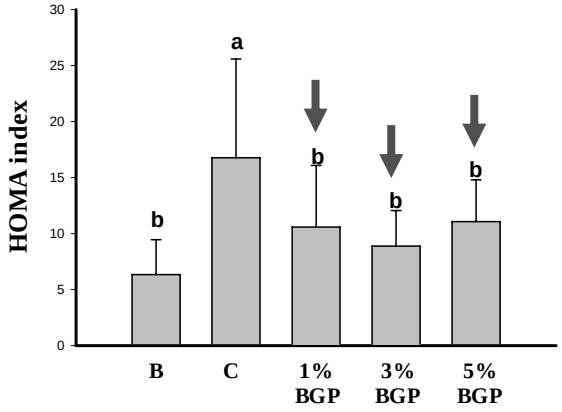
26

Area Under Curve of the OGTT



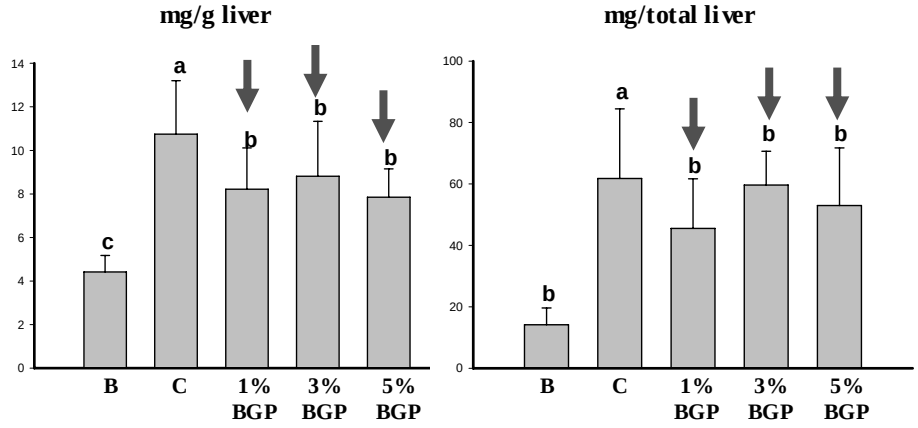
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HOMA index



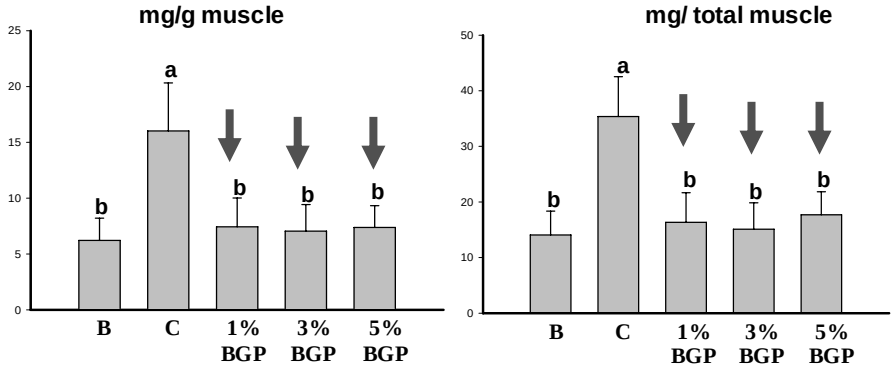
28

Liver cholesterol contents after 15 weeks of test diets feeding



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Muscle TG contents after 15 weeks of test diets feeding



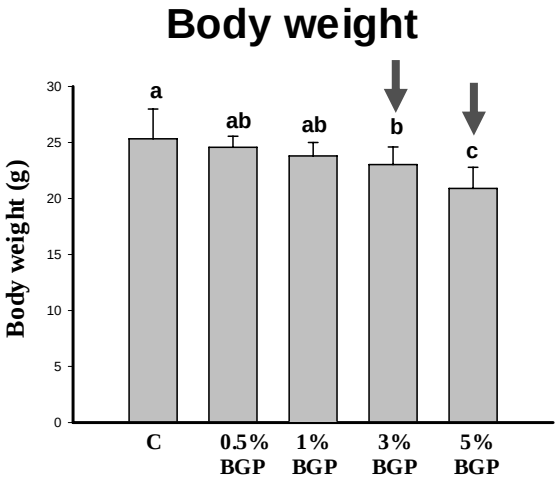
30

Experimental III: Dose-Response study

B: Freeze-Dried Wild Bitter Gourd diet effects on C57BL/6J mice

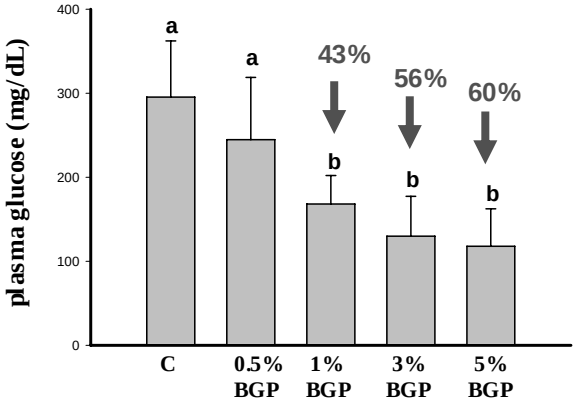


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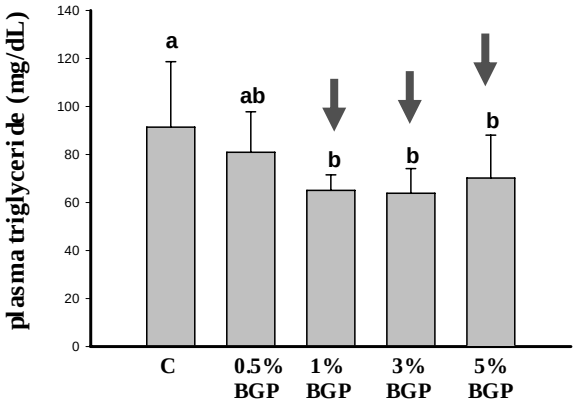
32

Plasma glucose concentrations



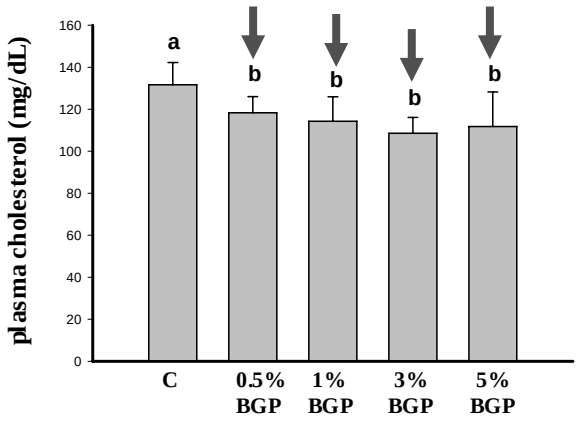
33

Plasma TG contents



34

Plasma CHOL concentrations



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Conclusion

- **1% bitter gourd diet significantly prevented**
  - **High fat diet induced obesity (Wistar rats)**
    1. **Hyperinsulinemia**
    2. **Glucose intolerance**
    3. **Liver CHOL accumulation**
    4. **Muscle TG accumulation**
  - **C57BL/6J**
    1. **Hyperglycemia**
    2. **Hypertriglyceridemia**
    3. **Hypercholesterolemia**
- **1% bitter gourd diet**
  ≈ 4.5 **g dry bitter gourd powder/day**
  ≈ 75 g fresh **bitter gourd / day**

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summary

	Improved exp.		Prevented exp.			Dose-dependent exp.	
	C57BL/6J		Wistar		C57BL/6J	Wistar	C57BL/6J
	5% BGP	0.25% EAE	5% BGP	1% EAE	5% BGP	1% BGP	
Body weight	↓	-	-	-	↓	-	-
Hyperglycemia	↓	-	↓	-	↓	↓	↓
Glu. intolerance	↓	-	↓	-		↓	
Hyperinsulinemia	-	-	↓	-	↓	-	
Hypertryglyceridemia	↓		-	↓	-	-	↓
Hypercholesterolemia	↓	-	-	-	-	-	↓
Liver TG accumulation	↓	↓	-	-		-	
Liver CHOL accumulation	-	-	-	-		↓	
Muscle TG accumulation						↓	

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Liver damage index: GOT and GPT

Group	GOT (U/l)	GPT (U/l)
Contorl	32.2 ± 15.4 [b]	11.3 ± 3.5 [ab]
5% BGP	39.7 ± 11.0 [b]	16.6 ± 6.6 [a]
mEAE	33.9 ± 10.7 [b]	10.6 ± 2.6 [b]
hEAE	65.3 ± 31.1 [a]	9.4 ± 4.9 [b]
Clofibrate	28.1 ± 5.6 [b]	10.2 ± 1.5 [b]
Rosiglitazone	40.7 ± 11.9 [b]	10.6 ± 1.0 [b]

Normal range:

GOT = 50~100 U/l

GPT =20~50 U/l

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吃得苦中苦，方為人上人

Purification and structural determination of bioactive constituents from *Momordica charantia*

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ABSTRACT

Five new cucurbitane-type triterpenes, (23*E*)-25-methoxycucurbit-23-ene -3β, 7β-diol (**1**), (23*E*)-cucurbita-5,23,25-triene-3β,7β-diol (**2**), (23*E*)-25-hydroxycucurbita -5,23-diene-3,7-dione (**3**), (23*E*)- cucurbita-5,23,25-triene-3,7-dione (**4**), and (23*E*)- 5β,19-epoxycucurbita-6,23-diene-3β,25-diol (**5**) together with five known triterpene, (23*E*)-5β,19-epoxy-25-methoxycucurbita-6,23-dien -3β-ol (**6**), 3β,25-dihydroxy-7β-methoxycucurbita-5,23(*E*)-diene (**7**), 3β -hydroxy-7β,25-dimethoxycucurbita-5, 23(*E*)-diene (**8**), 3β,7β,25-trihydroxycucurbita-5,23(*E*)-dien-19-al (**9**), and 25- methoxy-3β,7β-dihydroxycucurbita-5,23(*E*)-dien-19-al (**10**) have been isolated from the methanol extract of the stems of *Momordica charantia*. The structures of the new compounds were elucidated by spectroscopic methods.

Key words：*Momordica charantia;* stem; triterpene; cucurbitane

Momordica charantia L. (Cucurbitaceae), a slender-stemmed tendril climber, is wildly cultivated as a vegetable crop and has been used extensively in folk medicine as a remedy for diabetes in Asia. Previous investigations have shown that crude extracts of the fruit of *M. charantia* possess antidiabetic activity[1,2] and many cucurbitane-type triterpenoids have been isolated from the fruits,[3-9] seeds,[10-12] and leaves and vines[13] of *M. charantia*. Based on an interest in the discovery of bioactive natural products, we have continued with our efforts to elucidate the antidiabetic components from Taiwanese *M. charantia*. We have examined the methanolic extract of the stems of this plant and have isolated five new cucurbitane-type triterpenoids (compounds **1-5**), together with five known cucurbitane-type triterpene (compound **6-10**).[5,14,15] In this paper, we report the extraction, isolation, purification, and structural elucidation of those new constituents based on spectroscopic analysis.

Results and Discussion

Compound **1** gave a positive Liebermann-Burchard test. The HREIMS of the ion peak at *m/z* 472.3916 $[M]^+$ was consistent with the molecular formula, $C_{31}H_{52}O_3$. The IR spectrum showed the presence of hydroxyl (3360 cm^{-1}) and double bond (1625 and 700 cm^{-1}) functionalities. The ^1H and ^{13}C NMR spectra of **1** (Table 1) indicated the presence of seven tertiary methyls [δ_H 0.69, 0.89, 1.01, 1.04, 1.19 (3H each, s), 1.23 (3H × 2, s)], one secondary methyl [δ_H 0.87 (3H, d, J = 6.6 Hz)], a methoxyl [δ_H 3.12 (3H, s)], and two oxymethines [δ_H 3.52 (1 H, br s), 3.92 (1H, d, J = 5.6 Hz)]. In addition, olefinic NMR signals of a trisubstituted double bond [δ_H 5.80 (1H, d, J = 5.6 Hz); δ_C 122.6 (d), 146.8 (s)] and a *trans*-oriented disubstituted double bond [δ_H 5.36 (1H, d, J = 15.6 Hz), 5.48 (1H, ddd, J = 15.6, 8.4, 5.6 Hz); δ_C 128.5 (d), 136.7 (d)] coupling to a neighboring methylene [δ_H 1.76 m, 2.16 m; δ_C 39.4 (t)] were also found. The ^{13}C NMR spectrum of **1** revealed 31 carbon signals, which were assigned by DEPT experiments as nine methyls, seven methylenes, four methines, five quaternary carbons, four olefinic carbons, and two oxygenated carbons. By comparison of the ^1H and ^{13}C NMR data with the known compound, (23*E*)-3β-hydroxy-7β, 25-dimethoxycucurbita-5,23-dien-19-al,[9] compound **1**

was considered as a cucurbitane-type triterpene with a Δ^{23} -unsaturated C-8 side chain moiety. The downfield shift of H-7 [δ_H 3.92 (1H, d, J = 5.6 Hz)] in **1** indicated the presence of a hydroxyl group at C-7 instead of 7$_\beta$-methoxy group. Moreover, the disappearance of aldehyde signal of C-19, along with the presence of one additional methyl carbon signal (δ_C 29.5) led to the proposal that the aldehyde group of (23*E*)-3β-hydroxy-7β, 25-dimethoxycucurbita-5,23-dien-19-al was reduced to a methyl group in **1**. The HMBC spectrum of **1** showed long-range correlations between H-3 (δ_H 3.52) and C-1 (δ_C 20.9) and C-5 (δ_C 146.8), and between H-7 (δ_H 3.92) and C-5 (δ_C 146.8), C-6 (δ_C 122.6), C-8 (δ_C 53.2), and C-9 (δ_C 33.9), and this suggested that two hydroxyl groups were located at C-3 and C-7, respectively. The olefinic proton H-6 (δ_H 5.80) also exhibited HMBC correlations with C-4 (δ_C 41.5), C-7 (δ_C 68.2), C-8 (δ_C 53.2), and C-10 (δ_C 38.5). Futhermore, the α orientation of H-7 was determined by the cross-peaks between H-7 (δ_H 3.92) and H-30 (δ_H 0.69) observed in the NOESY spectrum of **1**. From the above evidence, compound **1** was characterized as (23*E*)-25-methoxycucurbit-23-ene-3β,7β-diol. Complete ^{1}H and ^{13}C NMR chemical shifts were established by ^{1}H- ^{1}H COSY, HMQC, HMBC, and NOESY spectra.

The HREIMS of **2** showed a molecular ion peak at *m/z* 440.3625, corresponding to the molecular formula, $C_{30}H_{48}O_2$, and indicating seven degrees of unsaturation. The IR spectrum displayed absorptions for hydroxyl (3350 cm^{-1}), and terminal double bond (3080, 1650, 875 cm^{-1}) functionalities. The ^{1}H NMR spectrum of **2** (Table 1) showed resonances for five tertiary methyls [δ_H 0.69, 0.90, 1.03, 1.06, 1.20 (3H each, s)], a secondary methyl [δ_H 0.89 (3H, d, J = 6.4 Hz)], a vinylic methyl [δ_H 1.83 (3H, s)], two secondary oxygenated methines [δ_H 3.52 (1 H, t, J = 2.4 Hz), 3.92 (1H, d, J = 5.6 Hz)], and a terminal methylene [δ_H 4.83 (2H, br s)]. Besides, olefinic proton signals attributing to a trisubstituted double bond [δ_H 5.80 (1H, d, J = 5.6 Hz)] and a *trans*-oriented disubstituted double bond [δ_H 6.08 (1H, d, J = 15.6 Hz), 5.60 (1H, ddd, J = 15.6, 7.2, 7.2 Hz)] coupling to a neighboring methylene [δ_H 1.79 m, 2.22 m; δ_C 39.7 (t)] were also observed. Altogether, 30 carbon signals were observed in the ^{13}C NMR spectrum of **2** and were sorted into seven methyls,

seven methylenes, four methines, four quaternary carbons, six olefinic carbons, and two secondary oxygenated carbons. The ^{13}C NMR data were very similar to those of **1**, except for the signals of C-17–C-23 of the side chain. A (23*E*)-$\Delta^{23,25}$-conjugated diene C-8 moiety as the side chain of **2** was suggested by both the NMR data [δ_H 4.83 (2H, br s, H-27), 5.60 (1H, ddd, J = 15.6, 7.2, 7.2 Hz), 6.08 (1H, d, J = 15.6 Hz); δ_C 114.0 (C-27), 129.4 (C-23), 134.1 (C-24), 142.2 (C-25)] and the fragment ions in the EIMS at *m/z* 81 $[CH_2CHCHC(CH_3)CH_2]^+$, *m/z* 109 [side chain]$^+$, *m/z* 331 [M－side chain]$^+$. In a HMBC experiment, correlations between H-23 (δ_H 5.60) and C-20 (δ_C 36.6), C-22 (δ_C 39.7), C-25 (δ_C 142.2) and H-27 (δ_H 4.83) and C-23 (δ_C 129.4), C-25 (δ_C 142.2), C-26 (δ_C 18.7) were observed. The relative configurations of methyl groups and other protons in the tetracyclic rings were determined by significant NOE correlations between H-3 (δ_H 3.52) and H-28 (δ_H 4.83), H-7 (δ_H 3.92) and H-30 (δ_H 0.69), H-30 (δ_H 0.69) and H-17 (δ_H 1.46) in the NOESY spectrum. Thus, compound **2** was elucidated as (23*E*)-cucurbita-5,23,25-triene-3β,7β-diol.

By HREIMS, compound **3** revealed a molecular formula of $C_{30}H_{46}O_3$ from the molecular ion at *m/z* $[M]^+$ 454.3423, indicating the presence of eight degrees of unsaturation. The IR spectrum showed absorption bands at 3400 cm^{-1} (hydroxyl), 1710 cm^{-1} (isolated ketone), and 1655 cm^{-1} (conjugated ketone). The UV spectrum displayed an absorption maximum at 256 nm. The ^1H and ^{13}C NMR spectra of **3** (Tables 1 and 2) exhibited seven tertiary methyls [δ_H 0.86, 0.87, 0.93, 1.31, 1.33 (3H each, s) and 1.28 (3H × 2, s)] and a secondary methyl [δ_H 0.88 (3H, d, J = 6.6 Hz)], and a α-H of α,β-unsaturated carbonyl system [δ_H 6.14 (1H, d, J = 2.4 Hz); δ_C 125.4 (d), 167.6 (s), 202.4 (s)]. Unambiguous comparison of ^1H and ^{13}C NMR data between **1** and **3** revealed that two oxymethines at C-3 and C-7 in **1** were absent. Instead, an isolated ketone and a α,β-unsaturated carbonyl carbons at δ_C 211.6 (C-3) and δ_C 202.4 (C-7) and a low field shifted carbon at δ_C 38.1 (C-2) were observed, indicating that **3** is an oxidized derivative of **1**. The isolated ketone and α,β-unsaturated carbonyl carbons were respectively assigned at C-3 and C-7, which were supported by the HMBC correlations between H-2 (δ_H 2.50, 2.58) and C-1 (δ_C

23.6), C-3 (δ_C 211.6), and C-10 (δ_C 41.2), and between H-6 (δ_H 6.14) and C-4 (δ_C 51.4), C-7 (δ_C 202.4), C-8 (δ_C 59.2), and C-10 (δ_C 41.2). ^{1}H-^{1}H COSY, HMQC, and HMBC analysis, together with the fragment ion at *m/z* 355 [M − $CH_2CHCHC(CH_3)_2OH$] $^+$ confirmed the side chain to be $CH_2CHCHC(CH_3)_2OH$. Compound **3** was accordingly determined as (23*E*)-25-hydroxycucurbita-5,23-diene-3,7-dione.

The molecular formula of compound **4** was assigned as $C_{30}H_{44}O_2$, on the basis of the HREIMS ($[M]^+$ *m/z* 436.3342), ^{13}C NMR, and DEPT spectra. Analysis of the IR spectrum of **4** suggested that it contains an isolated ketone (1715 cm^{-1}), a conjugated ketone (1665 cm^{-1}), and a conjugated terminal double bond (3085, 1655, 880 cm^{-1}). The UV spectrum exhibited an absorption maximum at 258 nm. ^{1}H and ^{13}C NMR data (Tables 1 and 2) of the tetracyclic part of the triterpene skeleton were almost the same as compound **3**, but the data in the the side chain were very similar to that of **2**. The proposed structure was also supported by the fragment ion peaks in the EIMS at *m/z* 81 $[CH_2CHCHC(CH_3)CH_2]^+$, *m/z* 109 [side chain]$^+$, *m/z* 327 [M−side chain]$^+$, and *m/z* 355 [M − $CH_2CHCHC(CH_3)CH_2]^+$. Thus, compound **4** was elucidated as (23*E*)-cucurbita-5,23,25-triene-3,7-dione.

Compound **5** was deduced to be a triterpenoid due to a positive Liebermann- Burchard test and was assigned the molecular formula of $C_{30}H_{48}O_3$, on the basis of molecular ion peak at *m/z* 456.3588 in the HREIMS. The IR spectrum of **5** showed bands attributable to hydroxyl group (3360 cm^{-1}) and double bond (1623 and 700 cm^{-1}) functionalities. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) indicated the presence of six tertiary methyls [δ_H 0.87, 1.18 (3H each, s), 0.84, 1.29 (3H × 2, s)], a secondary methyl [δ_H 0.87 (3H, d, J = 6.6 Hz)], an oxomethylene [δ_H 3.50 (1H, d, J = 8.4 Hz), 3.65 (1H, d, J = 8.4 Hz); δ_C 79.8 (t)], and a multiplet oxymethine [δ_H 3.36 (1H, $W_{1/2}$ = 6.6 Hz, H-3)] coupling to a hydroxyl group [δ_H 3.98 (1H, d, J = 9.6 Hz); disappeared on D_2O exchange]. In addition, the NMR signals for an allylic ABX system of *cis*-oriented cyclohexene [δ_H 6.02 (1H, dd, J = 9.6, 1.6 Hz, H-6), 5.61 (1H, dd, J = 9.6, 3.6 Hz, H-7), 2.32 (1H, br s); δ_C 131.9 (d), 131.5 (d), 52.0 (d)] were also found. The above NMR data of **5** were very similar to that of the known

compound, (23*E*)-5β,19-epoxy-25-methoxycucurbita-6,23-diene-3β-diol (**6**),[14] except that the methoxy group being replaced by a hydroxyl group at C-25. The structure of the side chain of **5** was also supported by the base peak of EIMS at *m/z* 109 ([side chain-H_2O]$^+$). Thus, compound **5** was determined as (23*E*)-5β,19-epoxy-cucurbita-6, 23-diene-3β,25-diol. Although compound **5** has been obtained by the hydrolysis of momordicoside I,[3] it was isolated for the first time as a natural product in the present investigation.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-180 digital spectropolarimeter. UV spectra were measured in MeOH on a Shimadzu UV-1601PC spectrophotometer. IR spectra were recorded on a Nicolet 510P FT-IR spectrometer. NMR spectra were recorded in $CDCl_3$ at room temperature on a Varian Mercury plus 400 NMR spectrometer, and the solvent resonance was used as internal shift reference (TMS as standard). The 2D NMR spectra were recorded using standard pulse sequences. EIMS and HREIMS were recorded on a Finnigan TSQ-700 and a JEOL SX-102A spectrometer, respectively. TLC was performed by using silica gel 60 F_{254} plates (200 μm, Merck). CC was performed on silica gel (230－400 mesh ASTM, Merck). HPLC was performed by using a Lichrosorb silica gel 60 (5 μm) column (250 × 10 mm).

Plant Material. The stems of *Momordica charantia* were collected in Ping-Tung, Taiwan in July, 2003. The plant material was identified by Prof. Sheng-Zehn Yang, Department of Forestry, National Pingtung University of Science and Technology. A voucher specimen (no. 2013) has been deposited at the Herbarium of this same institution.

Extraction and Isolation. Air-dried pieces of the stems of *M. charantia* (18 kg) were extracted three times with methanol (30 L) at room temperature (7 days each). The MeOH extract was evaporated in vacuo to afford a black residue, which was suspended in H_2O (3 L), and then partitioned sequentially

using EtOAc and *n*-BuOH (2L × 3). The EtOAc fraction (386 g) was chromatographed over silica gel, using mixtures of *n*-hexane and EtOAc of increasing polarity as eluents. Eleven fractions were collected as follows: fr. 1 [5000 mL, *n*-hexane], fr. 2 [4000 mL, *n*-hexane－EtOAc (49:1)], fr. 3 [4000 mL, *n*-hexane－EtOAc (19:1)], fr. 4 [4000 mL, *n*-hexane－EtOAc (9:1)], fr. 5 [4000 mL, *n*-hexane－EtOAc (17:3)], fr. 6 [4000 mL, *n*-hexane－EtOAc (8:2)], fr. 7 [4000 mL, *n*-hexane－EtOAc (7:3)], fr. 8 [3000 mL, *n*-hexane－EtOAc (5:5)], fr. 9 [3000 mL, *n*-hexane－EtOAc (4:6)], fr. 10 [(3000 mL, *n*-hexane－EtOAc (2:8)), fr. 11 (6000 mL, EtOAc). Fraction 5 was further chromatographed on a silica gel column (7×45 cm), eluted with *n*-hexane－CH_2Cl_2－EtOAc (8:6:1) to yield eight fractions (each 700 mL), fr. 5A-fr. 5H. HPLC of fr. 5D with *n*-hexane－EtOAc (8:2) as eluent, 2 mL/min, afforded **6**[14] (16 mg, t_R = 12.2 min) and **5** (12 mg, t_R = 21.3 min), respectively. Fraction 6 was further purified on a silica gel column (5×45 cm) eluted with CH_2Cl_2－EtOAc (8:1). Seven fractions (each 700 mL) were obtained as follows: fr. 6A-fr. 6G. HPLC of fr. 6E with *n*-hexane－CH_2Cl_2－EtOAc (8:6:1) as eluent, 2 mL/min, and afforded **2** (10 mg, t_R = 15.6 min) and **1** (8 mg, t_R = 18.2 min), respectively. Fraction 7 was further chromatographyed on a silica gel column (5×45 cm), eluted with CH_2Cl_2－EtOAc (8:1), to generate seven fractions (each 600 mL), fr. 7A-fr. 7G. HPLC of fr. 6F with *n*-hexane－EtOAc (17:3) as eluent, 2 mL/min, afforded **4** (9 mg, t_R = 13.5 min) and **3** (12 mg, t_R = 20.2 min), respectively.

(23*E*)-25-Methoxycucurbit-23-ene-3β,7β-diol (1): amorphous white powder; $[\alpha]^{25}_D$ +86.6 (*c* 0.4, $CHCl_3$); IR (KBr) ν_{max} 3360, 1625, 1463, 1379, 1242, 1191, 1131, 1021, 957, 825, 700 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 472 $[M]^+$ (3), 454 (14), 422 (27), 389 (100), 185 (12), 171 (13), 109 (15); HREIMS *m/z* 472.3916 (calcd for $C_{31}H_{52}O_3$ 472.3819).

(23*E*)-Cucurbita-5,23,23-triene-3β,7β-diol (2): amorphous white powder; $[\alpha]^{25}_D$ +81.5 (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} 3350, 3080, 1650, 1463, 1379, 1264, 1241, 1151, 1081, 1062, 953 , 875 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 440 $[M]^+$ (1), 422 (18), 407 (14), 389 (18), 325 (5), 135 (19),

109 (30), 81 (100); HREIMS *m/z* 440.3625 (calcd for $C_{30}H_{48}O_2$ 440.3642).

(23*E*)-25-Hydroxycucurbita-5,23-diene-3,7-dione (3): amorphous white powder; $[\alpha]^{25}_D$ +88.3 (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} 3400, 1710, 1655, 1467, 1338, 1224, 1152, 1076, 958, 735 cm^{-1}; UV (MeOH) λ_{max} (log ε) 202 (4.30), 256 (3.76) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 454 $[M]^+$ (1), 355 (22), 205 (13), 121 (100), 95 (82); HREIMS *m/z* 454.3423 (calcd for $C_{30}H_{46}O_3$ 454.3435).

(23*E*)-Cucurbita-5,23,25-triene-3,7-dione (4): amorphous white powder; $[\alpha]^{25}_D$ +97.6 (*c* 0.4, $CHCl_3$); IR (KBr) ν_{max} 3085, 1715, 1665, 1655, 1554, 1458, 1363, 1128, 1068, 998, 880, 737 cm^{-1}; UV (MeOH) λ_{max} (log ε) 202 (4.35), 258 (3.76) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 436 $[M]^+$ (5), 393 (5), 355 (25), 327 (8), 149 (37), 121 (100), 79 (75); HREIMS *m/z* 436.3342 (calcd for $C_{30}H_{44}O_2$ 436.333).

(23*E*)-5β,19-Epoxy-cucurbita-6,23-diene-3β,25-diol (5): amorphous white powder; $[\alpha]^{25}_D$ −82.2 (*c* 0.3, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 2; IR (KBr) ν_{max} 3360, 1623, 1457, 1375, 1236, 1160, 1035, 983, 851, 778, 700 cm^{-1}; EIMS *m/z* 456 $[M]^+$ (8), 438 (45), 390 (80), 309 (100), 281 (65); HREIMS *m/z* 456.3588 $[M]^+$ (calcd for $C_{30}H_{48}O_3$ 456.3605).

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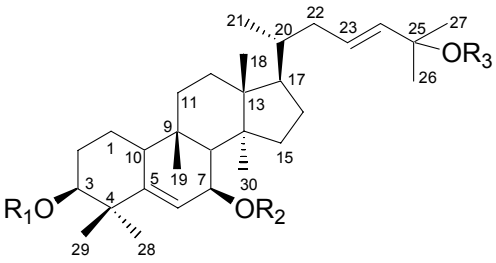
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Table 1. ^{1}H NMR Data for **1-5** (400 MHz in $CDCl_3$)

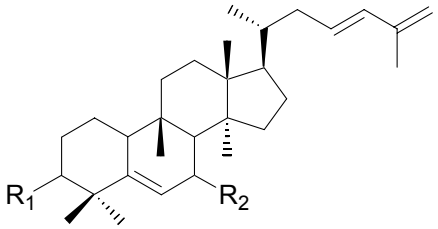
position	**1**	**2**	**3**	**4**	**5**
1	1.48 m, 1.56 m	1.50 m, 1.60 m	1.62 m, 2.10 m	1.63 m, 2.10 m	1.45 m
2	1.86 m, 1.72 m	1.72 m, 1.88 m	2.50 m, 2.58 m	2.51 m, 2.59 m	1.82 m
3	3.52 br s	3.52 t (2.4)			3.36 m
6	5.80 d (5.6)	5.80 d (5.6)	6.14 d (2.4)	6.14 d (2.4)	6.02 dd (1.6, 9.6)
7	3.92 d (5.6)	3.92 d (5.6)			5.61 dd (9.6, 3.6)
8	1.98 br s	2.00 br s	2.41 s	2.41 s	2.32 br s
10	2.28 m	2.29 m	2.89 m	2.90 m	2.24 dd (2.8)
11	1.42 m, 1.62 m	1.46 m, 1.62 m	1.54 m, 1.77 m	1.56 m, 1.78 m	1.46 m, 1.80 m
12	1.47 m, 1.64 m	1.48 m, 1.62 m	1.60 m, 1.74 m	1.62 m, 1.78 m	1.64 m
15	1.28 m, 1.32 m	1.28 m, 1.33 m	1.08 m, 1.55 m	1.10 m, 1.56 m	1.35 m
16	1.32 m, 1.88 m	1.40 m, 1.90 m	1.38 m, 1.86 m	1.42 m, 1.90 m	1.42 m, 2.00 m
17	1.44 m	1.46 m	1.42 m	1.50 m	1.48 m
18	0.89 s	0.90 s	0.87 s	0.88 s	0.84 s
19	1.04 s	1.06 s	0.93 s	0.92 s	3.50 d (8.4), 3.65 d (8.4)
20	1.48 m	1.32 m	1.54 m	1.52 m	1.45 m
21	0.87 d (6.6)	0.89 d (6.4)	0.88 d (6.6)	0.89 d (6.6)	0.87 d (6.6)
22	1.76 m, 2.16 m	1.79 m, 2.22 m	1.72 m, 2.12 m	1.78 m, 2.21 m	1.80 m, 2.14 m
23	5.48 ddd (15.6, 8.4, 5.6)	5.60 ddd (15.6, 7.2, 7.2)	5.57 m	5.59 m	5.57 m
24	5.36 d (15.6)	6.08 d (15.6)	5.57 m	6.09 d (15.6)	5.57 m
26	1.23 s	1.83 s	1.28 s	1.81 s	1.29 s
27	1.23 s	4.83, br s	1.28 s	4.83 br s	1.29 s
28	1.19 s	1.20 s	1.33 s	1.33 s	1.18 s
29	1.01 s	1.03 s	1.31 s	1.31 s	0.87 s
30	0.69 s	0.69 s	0.86 s	0.86 s	0.84 s
OCH_3	3.12 s				
OH					3.98 d (9.6)

Table 2. ^{13}C NMR Data for **1-5** (75 MHz in $CDCl_3$)

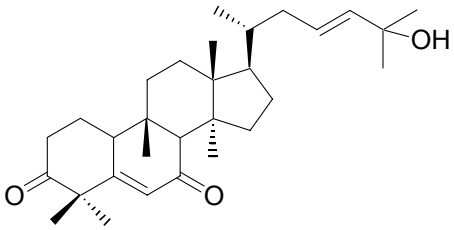
position	**1**	**2**	**3**	**4**	**5**
1	20.9	21.0	23.6	23.6	17.6
2	28.6	28.7	38.1	38.1	27.3
3	76.7	76.7	211.6	211.6	76.1
4	41.5	41.5	51.4	51.4	37.2
5	146.8	146.7	167.6	167.6	87.5
6	122.6	122.5	125.4	125.4	131.9
7	68.2	68.2	202.4	202.3	131.5
8	53.2	53.1	59.2	59.2	52.0
9	33.9	33.9	36.8	36.8	45.4
10	38.5	38.6	41.2	41.2	38.8
11	32.4	32.5	31.3	31.3	23.5
12	30.0	30.0	29.7	29.7	30.8
13	45.9	45.9	48.5	48.5	45.4
14	48.2	48.2	45.7	45.8	48.8
15	34.6	34.6	34.5	34.5	33.1
16	27.7	27.8	27.7	27.8	28.0
17	49.9	50.1	49.4	49.6	50.0
18	15.4	15.4	15.4	15.4	14.9
19	29.5	29.6	27.2	27.2	79.8
20	36.1	36.6	36.2	36.6	36.1
21	18.7	18.8	18.7	18.9	18.6
22	39.4	39.7	39.0	39.6	39.1
23	128.5	129.4	125.0	129.0	125.1
24	136.7	134.1	139.6	134.3	139.6
25	74.8	142.2	70.7	142.1	71.0
26	25.8	18.7	30.0	18.7	29.8
27	26.1	114.0	29.9	114.2	29.9
28	25.4	25.4	23.0	23.0	20.5
29	27.7	27.7	28.4	28.4	24.5
30	17.7	17.8	17.9	18.0	20.0
OCH_3	50.2				



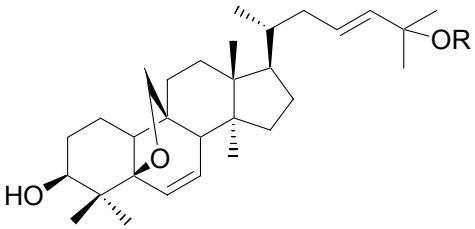
1 : R_1 = H, R_2 = H, R_3 = Me
7 : R_1 = H, R_2 = Me, R_3 = H
8 : R_1 = H, R_2 = Me, R_3 = Me



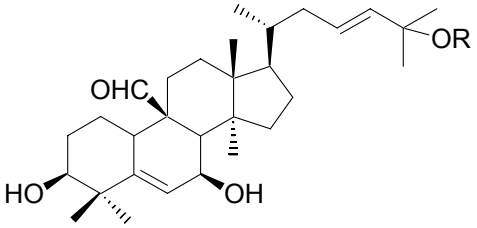
	R_1	R_2
2	β-OH	β-OH
4	= O	= O



3



5 R = H
6 R = CH_3



9 : R = H
10 : R = CH_3

苦瓜產業與保健功效研討會刊　　137-154

苦瓜萃物對動脈粥狀硬化及其相關因子的影響

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摘　要

苦瓜具有活化 PPARα及γ的成分，也含抗發炎成分。本研究探討苦瓜萃物或區分物對動脈粥狀硬化相關因子的影響。以容易產生動脈粥狀硬化之 apoE 剔除小鼠爲模式，由主動脈橫切觀察，發現苦瓜乙酸乙酯萃物可降低新生內層與中層比值，表對動脈粥狀硬化有抑制功能。在較年輕 apoE 小鼠，也觀察到苦瓜乙酸乙酯萃物可降低整條主動脈縱切的脂肪堆積。而以巨噬細胞爲模式，發現苦瓜乙酸乙酯區分物可抑制 LPS 誘發的 PGE2 產生，效果遠高於水萃物，且其成分爲含中短鏈脂肪酸之三酸甘油酯，脂肪酸中以 capric acid 抑制巨噬細胞產生 PGE2 能力最高。苦瓜之乙酸乙酯萃物可降低巨噬細胞產生 NO 及生成 MMP-9，降低人類主動脈內皮細胞生成 ICAM 及單核球細胞的粘附。總結：苦瓜乙酸乙酯萃物具有抗動脈粥狀硬化功能，可能經由抗發炎與降低白血球黏附到血管內皮細胞，對 MMP-9 生成的抑制也許有助動脈硬化斑塊的穩定。

關鍵詞：苦瓜、抗發炎、動脈粥狀硬化、巨噬細胞、內皮細胞

一、前言

國人代謝症候群的發生日益增多，包含胰島素抗性引起的葡萄糖不耐症、腹部肥胖、高 TG、低 HDL 與高血壓，是否轉變爲心血管疾病會受到體內未顯現臨床症狀的發炎程度影響。PPAR α 及 γ 活化劑分別可下降血脂與血糖，目前所用的降血脂藥物 fibrate 及降血糖藥物 glitazones 分屬之。PPAR- γ agonists，除可降血糖外，也會降低發炎反應，其分子機轉是活化 PPAR- γ 後抑制 monocyte 分泌細胞激素（Jiang et al, 1998; Ricote et al, 1998）。PPAR 亦有抗發炎的功能（Chinetti ey al, 2000）。血管壁上單核/巨嗜細胞、內皮細胞、上皮細胞與平滑肌細胞都會表現各種 PPARs，PPARs 被活化後，干擾 NF-kappaB, STAT, AP-1 訊息傳遞，而抑制發炎反應基因如 IL-1, IL-2, IL-6, IL-8, tumor necrosis factor- α （TNF- α ）, metalloproteases 表現（Chinetti et al, 2000），使 PPAR agonists 成爲治療動脈粥狀硬化藥物（Francis et al, 2003），尤其可同時活化兩種受器之藥物，被認爲具有最大之潛力。

苦瓜之乙酸乙酯萃取物具有相當於藥物 WY-14643 之 PPAR α 活化能力，促進肝臟 acyl-CoA oxidase 基因表現的成分，具增加肝臟脂肪酸氧化的作用[Chao et al, 2003]，預期具有透過 PPAR α 調節脂質代謝之能力。進一步測試，發現苦瓜萃取物對 PPAR γ 與 δ 也有極佳之活化能力。最近日本學者發現胃液消化過的苦瓜溶液會增高人類巨嗜細胞株 IL-10 分泌，IL-10 功能爲抑制 T 細胞功能，若以 5%凍乾苦瓜餵養大白鼠體外實驗，發現脾臟淋巴球細胞減少，故認爲苦瓜會抑制發炎（Manabe et al, 2003）。Huang 等人觀察到苦瓜萃取物可抑制小鼠腹腔巨噬細胞株在 LPS 刺激下 PGE2 之生成，可能具有抗發炎之功效（Huang et al, 2003）。

在動脈硬化的小動物模式中，apoprotein （apo）E deficient 小鼠 C57BL/6，因無清除血液中引起動脈粥狀硬化的大分子脂蛋白的能力，約 10 週大就會產生與人類動脈粥狀硬化相似的所有現象[Nakashima et al, 1994]，如血膽固醇高到 600 mg/dL，正常鼠僅 100 mg/dL，與人類 apoE2 突變型或糖尿病人常發生的 Type III hypercholesterolemia 類似，膽固醇與 TG 幾乎同高，於此模式也可見到 aortic root、aortic arch、pulmonary and carotid arteries 泡細胞堆積與損傷，PPAR agonist fenofibrate 可降低此種小鼠動脈粥狀硬化（Duez et al, 2002），人類粥瘤形成初期的發炎反應也可在此動物模式顯現，此種小鼠血清含有高濃度的抗各種被氧化的 LDL 的自

體抗體，有些可辨識人血清與粥狀斑上被氧化的 LDL（Palinski W, 1996）。動脈粥狀硬化的進展，無論是飲食引起或是基因缺損 （ApoE deficient, LDL receptor defect, apo B mutation）都相當類似（Reddick et al, 1994），因此以此爲動物模式。

本研究用 ApoE knockout mice 模式研究苦瓜對粥狀動脈硬化的改善效果，分析主動脈竇、主動脈竇後、胸主動脈三處的血管 Intima/Media area ratio，及主動脈縱切的脂肪堆積。爲了解山苦瓜降低動脈硬化的機轉與成分，以 RAW264.7 巨噬細胞爲模式，測試苦瓜乙酸乙酯萃物區分物，或不同的山苦瓜萃取物對 LPS 誘發的 PGE2、NO 等發炎因子，及使動脈粥狀硬化斑塊不穩定的 MMP-9 產生的抑制效果；並以人類主動脈內皮細胞爲模式，在 TNF α 刺激下，測試不同山苦瓜萃取物對細胞黏著分子表現與單核球化學趨化物質分泌的影響。

二、材料與方法

（一）材料

山苦瓜由行政院農業委員會花蓮區農業改良場提供，三種雜交品系（農友三苦瓜、花蓮一號、WB15）共約300公斤，全果直接送至彰化達群公司，洗淨後進行冷凍乾燥，得到17.6公斤凍乾粉末，產率爲5.9％。其中澱粉佔39.28％、可溶性糖8.65％、纖維38.2％、蛋白質4.51％、灰分6.7％、脂質2.66％。山苦瓜乙酸乙酯萃取物來自山苦瓜凍乾粉末5公斤浸泡於150公升乙酸乙酯，室溫下攪拌萃取24小時，再將萃取液於60℃進行減壓濃縮，得到250克，萃取率約5％（謝婉郁, 2005）。

（二）動物實驗

1.飼料

飼料的基本組成參考AIN-76（American Institute of Nutrition,1997）配方作修正，將玉米澱粉（Samyang Genex Corp,Seoul,Korea）和蔗糖（台糖精緻細砂）的比例改成1：1，其他成分包含酪蛋白（ICN Biomed）、甲硫胺酸（Sigma,USA）、纖維素（JRS. Vitacel,German）、AIN-76礦物質混和物（ICN Biomed）、AIN-76維生素混和物（ICN Biomed）、膽鹼（Sigma）、膽固醇（Hanawa,Japan）、紅花子油（台糖紅花子油）、奶油（安佳無水奶油），另外分別添加山苦瓜凍乾粉末、山苦瓜乙酸乙酯萃取物、Clofibrate

（Fluka）、TZD（Avandia）。依山苦瓜成分分析結果，當飼料中給予5 %山苦瓜粉末，將依成分比例取代飼料中starch （-2.9%）、cellulose（-2.1%）；而使用山苦瓜乙酸乙酯萃物1%則取代 1% butter；此外，也調整高脂飼料protein, mineral, vitamin, cellulose 之nutrition density，使其同於低脂飼料（chow diet）。實驗一使用6 種飼料：（1）chow diet：MF18商業錠狀飼料。（2）高脂：含20%油脂。（3）高脂苦瓜：含20%油脂＋5%山苦瓜凍乾粉末。（4）高脂乙酸乙酯萃物：含19%油脂＋1%乙酸乙酯萃取物。（5）高脂Clofibrate：含20%油脂＋0.5%Clofibrate。（6）高脂TZD：含20%油脂＋0.00123%TZD （rosiglitazone）。實驗二使用與前面相同之高脂、高脂苦瓜、高脂乙酸乙酯萃物三種飼料。

2.實驗動物

實驗一將18週齡的C57BL/6J apo E knockout的雄性小鼠45隻依體重隨機分6組，餵養18週；實驗二15隻10週、7隻9週、11隻6週齡的C57BL/6J apo E knockout的雄性小鼠共33隻，依體重、年齡分成三組，餵食8週。飼養期間，每日光照12小時，黑暗12小時，使用IVC cage（5隻/籠），每週更換高溫高壓滅菌籠2次，飼料及水自由攝取，飼料攝取量每週紀錄2次，體重每週紀錄1次，並視需要由眼窩採血，犧牲後收集主動脈血管。

3.主動脈橫切面之新生內層及中層面積比

將取下之主動脈弓（aortic arch）及胸主動脈（thoracic aorta）旁的脂肪組織剔除乾淨後，以含4%福馬林（paraformadehyde）之PBS固定液進行固定，更換兩次後處理隔夜，將經固定液固定完之心臟血管從左右頸動脈分支之動脈弓處分開，將兩段組織分別置於裝有新鮮的PBS的標本瓶1小時後（30鐘更換一次），再更換新的PBS保存於4℃至進行石蠟包埋（paraffin embedding），將包埋好的石蠟塊修爲梯型並固定於包埋盒座上，以切片機進行切片，含心臟和動脈弓前段之組織以7μm厚度、動脈弓後段血管組織以5μm厚度進行切片，將切出的片子，小心放入37～40℃水浴槽進行展片，每10片收取1片置於上有poly-L-lysine之玻片上，接著置於37℃烘片機中乾燥，採用Mayrt，s Hematoxylin & Eosin stain方法，將保存之切片進行蘇木精-伊紅染色（Hematoxylin & Eosin stain），接著，將已製作好的動脈組織切片，利用光學顯微鏡連接電腦影像分析系統Image-pro plus 2.0軟體 （Olymus Model-ck30）拍照並計算動脈組織橫切面之新生內層及中層面積比率Intima/Media Ratio（如附圖3-3-2），再進行統計比較之。

4.主動脈縱切與脂肪堆積染色

主動脈弓（aortic arch）及胸主動脈（thoracic aorta）以含4%福馬林（paraformadehyde）之PBS固定後，以PBS清洗後，浸泡於50%isopropanol 10分鐘，再以配置好的Oil red-O（5mg/ml in isopropanol）染色2分鐘，再以50% isopropanol 退染1分鐘，最後置於PBS內保存。經由Oil-red-O染色後，從血管腹面縱向剪開後，脂肪會呈現深紅色斑點，接著以照相系統（Olympus）進行拍照，最後使用ImageJ軟體（NIHI網站提供）分析主動脈前1公分長度硬塊面積與血管面積的比率。

（三）細胞實驗

1.RAW264.7 cell line之前列腺素（PGE2）、NO、IL-10、TNF-α分泌，及iNOS表現

待測苦瓜樣品以培養液（DMEM）稀釋爲不同濃度進行細胞實驗，培養18小時後，以MTT測細胞存活率，若實驗組之細胞數低於僅以培養液培養的控制組，則表示該濃度之樣品具細胞毒性。因此，決定培養之樣品濃度範圍以不影響細胞生長爲原則，以排除樣品濃度對細胞生長之干擾。以LPS 100 ng/ml 處理 RAW264.7 18小時誘導PGE2生成，觀察苦瓜乙酸乙酯萃取物之區分物對RAW264.7細胞株之PGE2生成量的影響，採用市售試劑套組以競爭性免疫分析方法進行PGE2分析，爲避免因細胞增生造成PGE2濃度的差異，故將每well所測得之PGE2生成量除以1 x 105單位細胞數表示之。IL-10、TNF-α以Elisa，iNOS用西方點墨法測量。

2.人類單核球細胞株THP-1之 MMP-9表現

THP-1購自食工所生資中心，使用RPMI 1640培養液（Gibco），內含2 μM L-glutamin、4.5g/L glucose、10Mm HEPES、1.0mM sodium pyruvate（SIGMA），外加fetal bovine serum 10%（Hyclone）及0.05mM 2-mercaptoethanol（SIGMA）。加入100 mg/ml PMA 培養THP-1 48 h 促進分化爲巨噬細胞，再加50 mg/ml ox-LDL及苦瓜萃物，收集細胞上清液後，採用市售Human MMP9 set （R&D Systems）進行分析。

3.人類主動脈內皮細胞表現ICAM-1、分泌MCP-1與NO生合成，以及被單核球黏附情況。

HAECs細胞株（Cascade Biologics）、培養液M200（Cascade Biologics）、LSGS（Cascade Biologics）、TE/TN（Cascade Biologics）、

PSF抗生素（GIBCO）、抗黴劑Methyl 4-hydroxybenzoate（SIGMA）、細胞級DMSO（SIGMA）、trypan blue（SIGMA）、液態氮（巧充）、fetal bovine serum（HyClone）。內皮細胞以TNF-α（2 ng/ml）刺激，同時與2381及CK兩品種山苦瓜之乙酸乙酯萃物、皂化與不皂化萃取物（EAE、S及NS）共同處理12小時。以Human CCL2/MCP-1 kit assay （R＆D Systems）分析MCP-1；以Griess Reagent System組（Promega）分析NO；抽取細胞蛋白質以西方點墨法（Western blotting）進行分析ICAM。內皮細胞/單核球黏附分析，先以螢光劑 （2’,7’ -bis（2-carboxyethyl）-5（6）-carboxyfluore- scein acetoxymethyl ester；Invitrogen,進階）標示U937細胞，再與內皮細胞在培養箱中共同培養一小時，以螢光光度計（TEKON Techonolgies）讀取Ex 485/Em 530螢光強度。

（四）統計方法：請見圖表。

三、結果

（一）動物實驗

實驗 1.胸主動脈 Intima / Media area ratio

高脂 Clofibrate 顯著高於 Chow diet、高脂 EA 和高脂苦瓜組，且 EA 組顯著低於高脂組。

表一、胸主動脈 Intima / Media area ratio (Exp. 1)

Table 1. The Intima / Media area ratio of thoracic aorta in apoE knockout mice fed six different diets

組別	n	Intima/Media Area ratio
Chow diet	5	0.230 ± 0.058^{bc}
高脂	10	0.739 ± 0.225^{ab}
高脂苦瓜	10	0.440 ± 0.114^{bc}
高脂 EA	10	0.320 ± 0.062^{c}
Clofibrate	5	0.999 ± 0.297^{a}
TZD	5	0.665 ± 0.123^{abc}

1.Values are Mean±SEM

2.Data were analyzed by one-way ANOVA LSD test. Values with different superscript letters are significantly different at $p<0.05$ level

實驗 2.主動脈脂肪堆積情形

經由 oil-red-O 染色觀察主動脈脂肪堆積情形，計算出主動脈損傷面積百分比，以 ANOVA LSD 多重比較法分析，高脂 EA 顯著低於高脂苦瓜組與高脂組（如表三）。

表二、主動脈損傷面積百分比

Table 2. Percent of aortic lesion areas in apoE knockout mice fed three different diets.

組別	n	Lesion areas (%)
高脂	11	11.51 ± 1.26^{a}
高脂苦瓜	10	13.04 ± 1.21^{a}
高脂 EA	10	8.35 ± 0.59^{b}

1.Values are Mean±SEM

2.Data were analyzed by ANOVA LSD test. Values with different superscript letters are significantly different at $p<0.05$ level

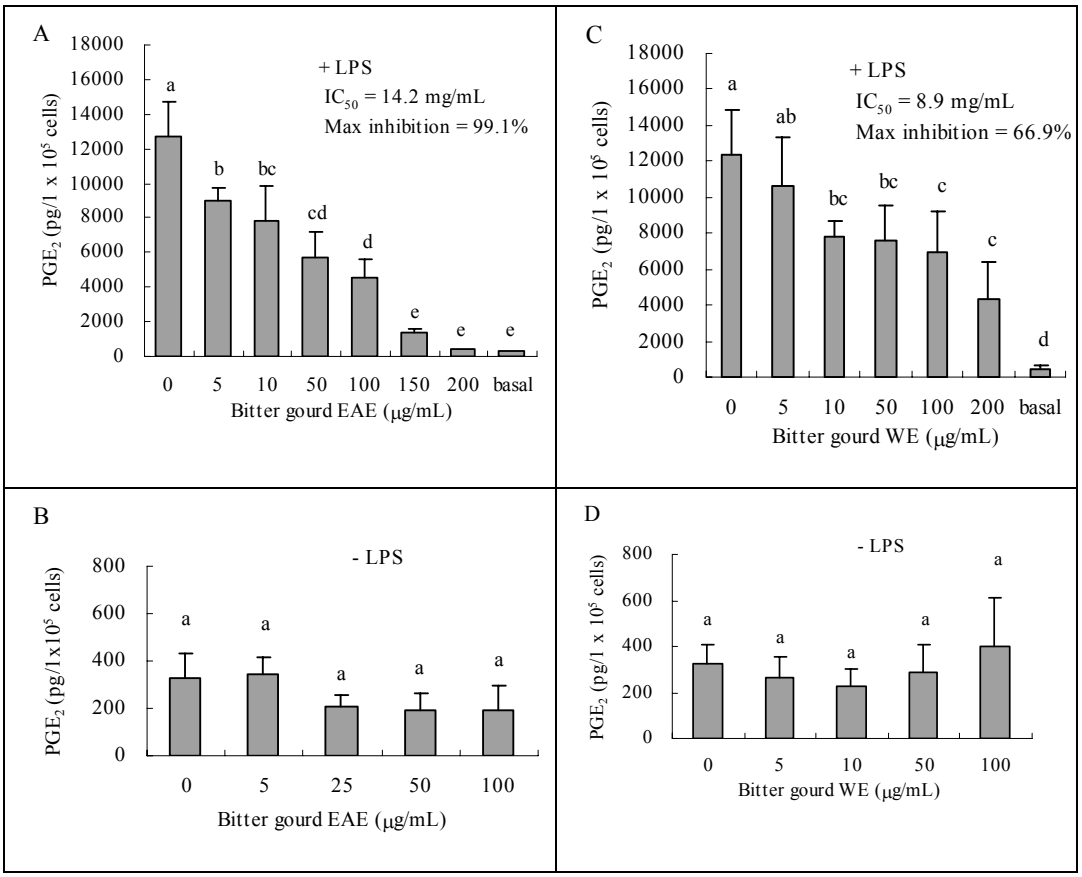
表三. 動物實驗 1 & 2 結果

Table3. the outcome of animal models(1&2)

	動物實驗 1	動物實驗 2
餵食劑量	20%高脂＋5%山苦瓜凍乾粉末 20%高脂＋1%山苦瓜乙酸乙酯萃物	20%高脂＋5%山苦瓜凍乾粉末 20%高脂＋1%山苦瓜乙酸乙酯萃物
開始實驗 age	18 週大	10 週(15 隻)、9 週(7 隻)、6 週(11 隻)大
餵食週數	18 週	8 週
體重變化	高脂苦瓜組在 2-7 週顯著低於與其他組，13 週後無顯著差異	高脂苦瓜組從第二週開始體重顯著低於高脂、高脂 EA 組
攝食量	高脂 EA 最高，高脂苦瓜組最低	高脂苦瓜組最高，高脂組最低
攝食效應	高脂 EA 最高，Clofibrate 組最低	高脂組最高，高脂苦瓜組最低
器官重	絕對與相對副睪脂高脂 EA 顯著高於 chow diet 組 絕對肝重高脂 Clofibrate 顯著高於高脂組	絕對腎重高脂苦瓜組顯著低於高脂與高脂 EA 組 相對脾重高脂苦瓜顯著高於高脂與高脂 EA 組
血清發炎因子	IL-1β、IL-6、TNFα 皆無顯著差異	IL-6、TNFα 皆無顯著差異
血清黏著分子	ICAM-1 高脂苦瓜顯著高於 chow diet，VCAM-1 無差異	無測量
白血球上 CD11b/CD18 表現	無測量	各組無顯著差異
血清 8-iso-PGF$_{2\alpha}$	無測量	各組無顯著差異
血管分析	胸主動脈切片高脂 EA 顯著低於高脂組	縱切高脂 EA 顯著低於高脂與高脂苦瓜組

（二）山苦瓜萃取物對 RAW264.7 之影響

1.乙酸乙酯萃取物(EAE)與 LPS 同時處理下，具有抑制 PGE2 生成的作用 (Fig 1-A)。乙酸乙酯萃取物(EAE)的抑制效果較於水萃物(WE)強 (Fig 1-C)。

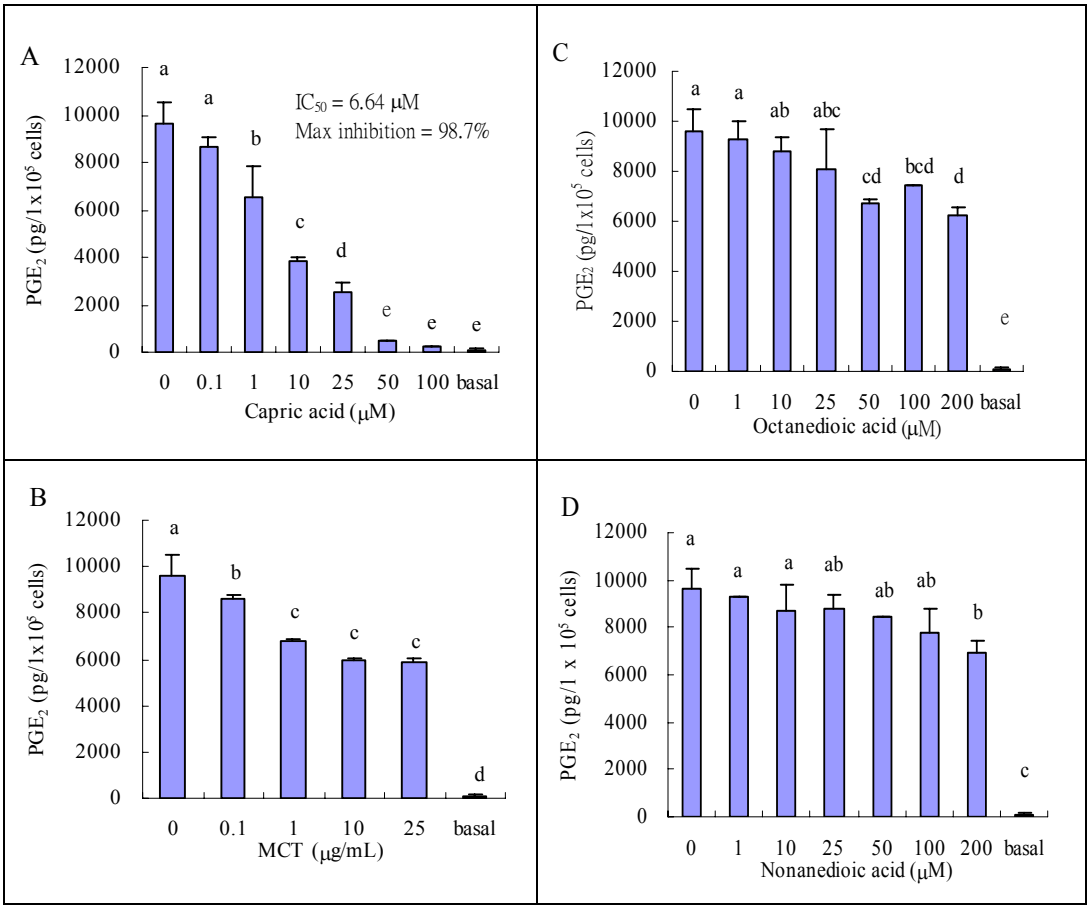


圖一、乙酸乙酯苦瓜萃物與水萃苦瓜對 RAW264.7 細胞 PGE_2 生成量之影響

Fig 1. Effects of ethyl acetate extract (EAE) (A, B) and water soluble extract (WE) (C, D) of bitter gourd on PGE_2 production in RAW264.7 cells. The cells were treated with various concentrations of the extracts in the presence (+LPS) or absence (-LPS) of LPS (100 ng/mL) for 18 hrs and then medium was collected for PGE_2 analysis using an EIA.

Basal: RAW264.7 cells incubated with medium only. 0: RW264.7 cells treated with LPS only.

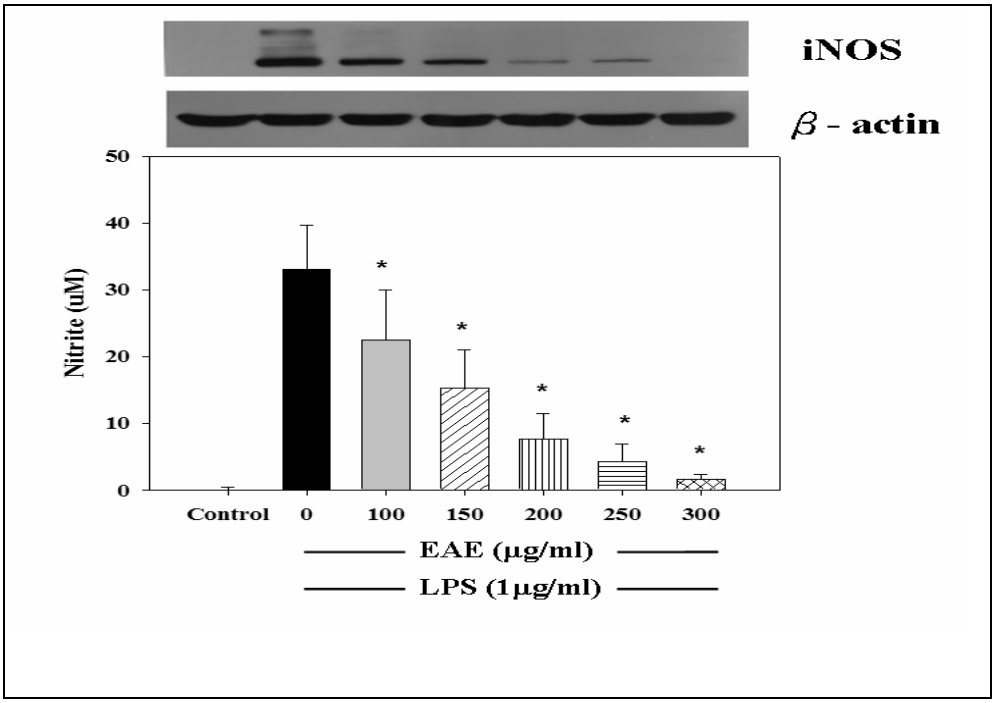
2.山苦瓜 EAE 經不同極性溶劑對萃、4 次 silica gel column chromatographies 與 reverse phase C-18 chromatography 得活性最高之 RP-10 區分物，再經 H-NMR 與 H-H COSY 分析發現爲含中短鏈脂肪酸之三酸甘油酯，以各種脂肪酸測試抑制 PGE2 合成能力，發現 capric acid 活性最高(Fig 2A)，MCT , octaredoic acid, nonaredoic acid 亦有較低能力 (Fig2B-D)。



圖二、capric acid (A), MCT (B), octanedioic acid (C)及 nonanedioic acid (D) 對 RAW264.7 細胞 PGE_2 生成量之影響

Fig 2.Effects of capric acid (A), MCT (B), octanedioic acid (C), and nonanedioic acid (D) on PGE_2 production in RAW264.7 cells. The cells were treated with various concentrations of fatty acids or MCT in the presence of LPS (100 ng/mL) for 18 hrs and then medium was collected for PGE_2 analysis using an EIA.

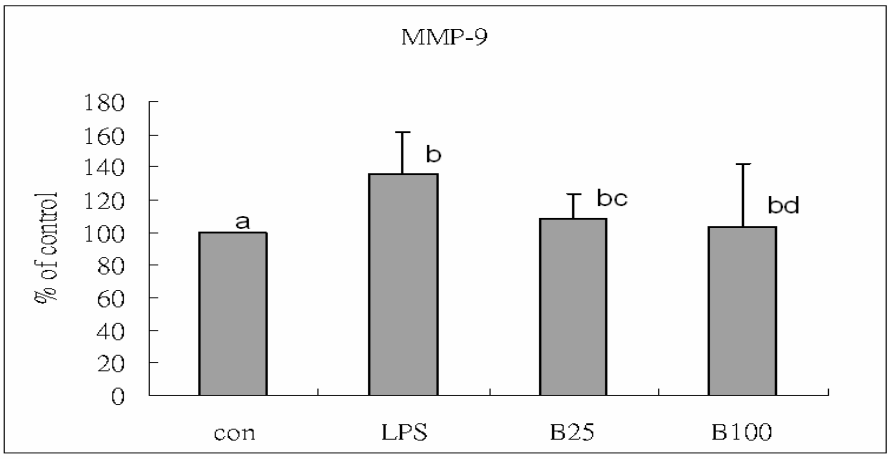
3.巨噬細胞產生大量 NO 是發炎的現象，苦瓜乙酸乙酯萃物可下降 LPS 刺激下 Raw264.7 的 iNOS 酵素活性與 NO 產生，且有劑量反應 (Fig 3)。



圖三、乙酸乙酯苦瓜萃物對 NO 生成量與 iNOS 表現量之影響

Fig 3.Effect of ethyl acetate extract (EAE) of wild bitter gourd on LPS-Induced nitric oxide production and iNOS expression in RAW 264.7 macrophage cells. $p < 0.05$ vs cells with LPS alone (0 g/ml of EAE).

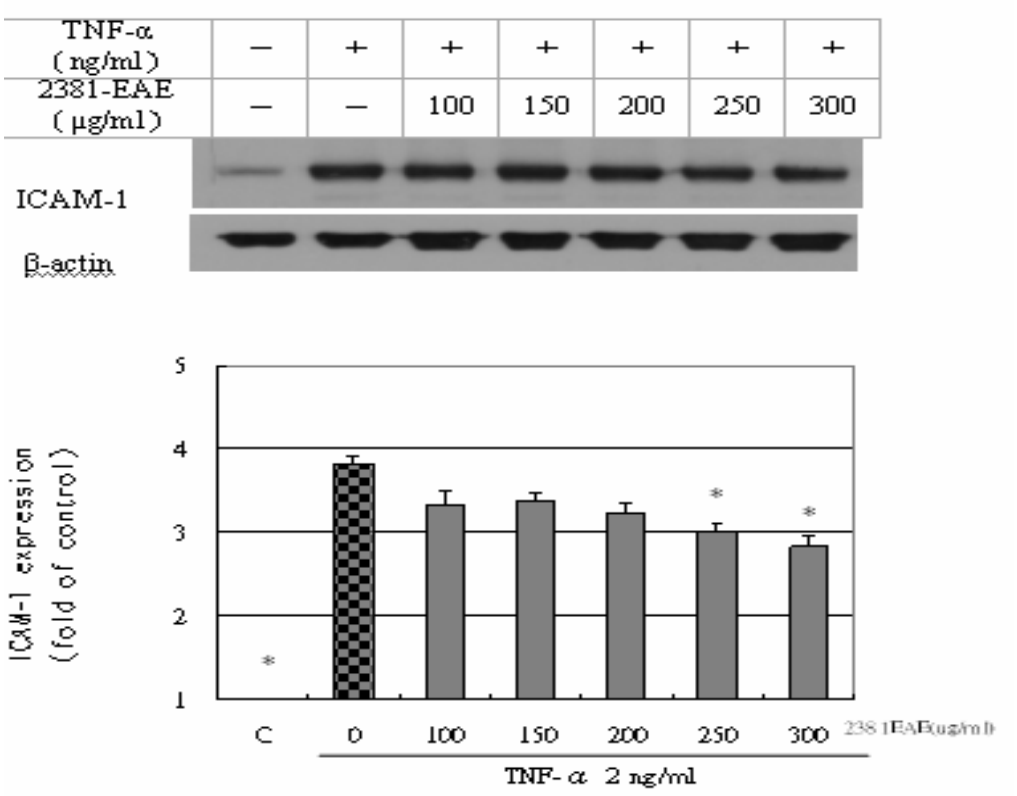
4.苦瓜萃物下降人類巨噬細胞株 THP-1 在 LPS 刺激下所產生的 MMP-9 (Fig 4)



PMA100ng/ml	+	+	+	+
LPS (10ng/ml)	—	+	+	+
Bitter gourd(μ g/ml)	—	—	25	100

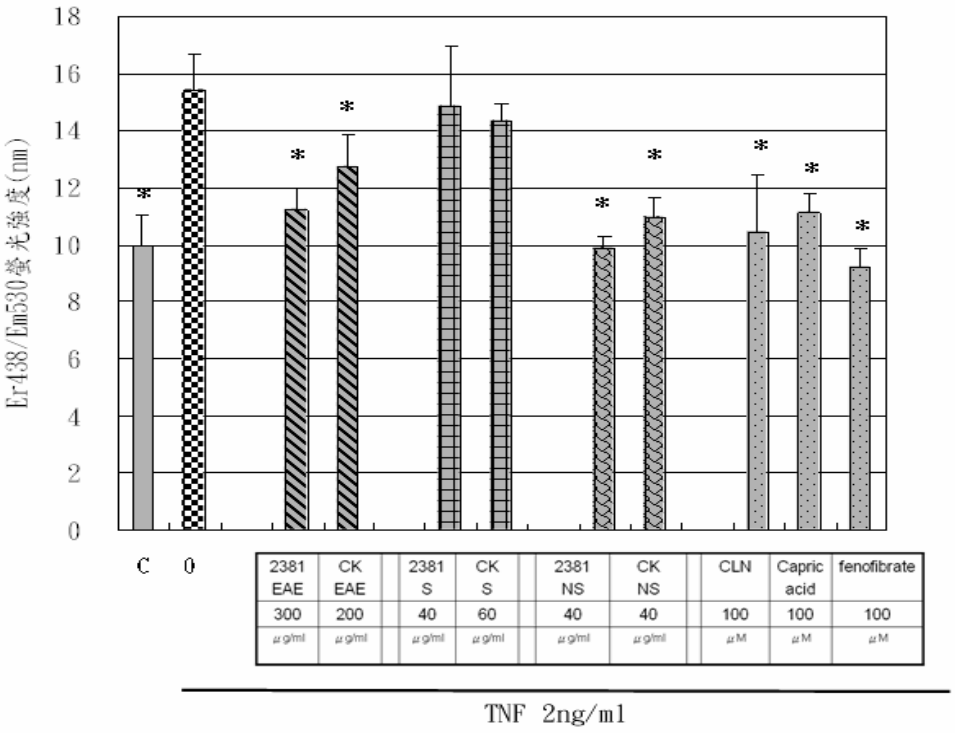
Fig 4.THP-1were treated with PMA 100ng/ml for 48hrs and then treated with bitter gourd EAE extract and LPS for 24hrs. Medium were collected and assayed for MMP9.

5.苦瓜萃物下降 TNFa 刺激下人類主動脈內皮細胞合成 ICAM (Fig 5)，及被單核球細胞粘著 (Fig 6)



圖五、苦瓜 2381 乙酸乙酯萃物對 ICAM-1 表現量之影響

Fig 5.Effects of wild bitter gourd 2381- ethylacetate extracts (2381-EAE) on ICAM-1 expression in TNF-α stimulated HAEC. Cells incubated without bitter gourd and TNF-α were the negative control (C). Amounts of ICAM-1 were quantified using β-actin as loading control and are expressed relative to those of control conditions. Values are means ± SD, n＝4.*Significantly different from 0 μg/ml analyzed by Mann-Whitney U test at $p < 0.05$.



圖六、苦瓜萃物對於細胞黏附作用之影響

Fig 6.Effects of extract of bitter gourd on the adhesion of U937 monocytes to TNFα-stimulated HAEC. Monocytes were labeled with 2 μM 2′,7′-bis -(2-carboxyethyl)-5-(and-6)-carboxyfluorescein acetoxymethylester (BCECF-AM, Molecular Probes). Fluorescence was read using a filter pair for excitation at 485 nm and emission at 530 nm. Cells incubated without bitter gourd and TNF-α were the negative control (C). Values are means ± SD, n＝3. *Significantly different from 0 μg/ml analyzed by Mann-Whitney U test at $p < 0.05$.

四、討論

經兩次獨立實驗分別發現苦瓜 EA 萃物可降低主動脈內皮層增厚程度 57%，及降低主動脈弓與胸主動脈脂肪堆積 28%，因此推論山苦瓜乙酸乙酯萃物具有改善動脈粥狀硬化之功效。山苦瓜凍乾粉末無法改善動脈硬化的原因可能因高脂苦瓜組的有效成分只有高脂 EA 組的 1/4。因爲前兩動物實驗，苦瓜 EA 萃物都無下降血脂，故探討其他作用機轉包含抗發炎、降低白血球粘結到血管壁。

由巨噬細胞株實驗得知山苦瓜乙酸乙酯萃物有抗發炎成分，可能是含中短鏈脂肪酸的三酸甘油酯的成分，且第一次發現脂肪酸中 capric acid 具備最強抑制巨噬細胞產生 PGE2 的能力，此效果可能類似它的抗 gram(-)菌作用。苦瓜萃物也顯示其他抗發炎功能如降低巨噬細胞產生 NO，及使粥狀硬化斑塊不穩定的 MMP-9。在動脈硬化初期先有單核球粘著到動脈壁，才有泡細胞形成，其黏著需內皮細胞分泌黏著因子，若此步驟受到抑制將可延緩動脈粥化的發生與進展，本研究發現苦瓜萃物降低內皮細胞製造粘著因子 ICAM，並觀察到單核球細胞黏著到內皮的程度降低，這些結果可解釋苦瓜萃物改善動脈粥狀硬化的機轉，並可視爲篩選苦瓜品種與有效成分的工具。

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The effect of bitter gourd extracts on atherosclerosis and its related factors

ABSTRACT

Bitter gourd contains some bioactive constituents which can activate PPARα and γ induce anti-inflammatory responses. This study was to investigate the effect of bitter gourd extract on atherosclerosis and related factors, and identify its active components for functional food development. The results indicated that ethyl acetate extract of bitter gourd decreased intima/media area ratio of aorta in apoE knockout mice and the effect was consistent in young apoE knockout mice by decreasing en face lipid accumulation of aorta. To investigate the mechanisms and establish screening methods, several cell lines were used. Bitter gourd extract inhibited LPS induced PGE2 production in Raw 246.7, and triglycerides constituted of short chain and medium chain fatty acids were identified in the active fraction. Among 3-20 C fatty acid, capric acid was found to possess the highest PGE2 inhibition effect. LPS induced NO production in Raw264.7, LPS induced MMP-9 production in THP-1, and TNFa induced ICAM production in human aorta endothelial cell (HAEC) decreased by bitter gourd extract. Monocyte adhesion to TNFa stimulated HAEC also decreased. In conclusion, bitter gourd extract improves atherosclerosis through anti-inflammation and inhibiting the adhesion of monocytes to aorta endothelial cells, and potentially improves the stability of atherosclerotic plagues by decreasing the production of MMP-9 in macrophages.

Key words：bitter gourd, plasma lipids, anti-inflammation, atherosclerosis, macrophages

苦瓜產業與保健功效研討會刊　155-168

苦瓜對 HepG2 及 HepG2.2.15 肝癌細胞的抗病毒及抗腫瘤作用

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摘　要

爲了由天然資源中尋找具抗病毒及抗腫瘤活性的有效成分，過去已經有相當多的植物及食品組成進行評估。苦瓜（學名 *Momordica charantia*）因熟知其具有降血醣、抗醣尿、抗病毒及抗腫瘤之功能，而被廣泛應用於日常保健食材及傳統醫學上。爲了釐清不同品系苦瓜所具有的多項生物功能及作用情形，本研究乃以肝癌細胞株 HepG2 及感染 B 型肝炎病毒（HBV）的 HepG2.2.15 肝癌細胞株爲研究對象，同時進行苦瓜抗病毒及抗腫瘤的活性評估。苦瓜處理的細胞是以 MTT 個別進行細胞毒性的比較分析，在所有以乙醇或二氯甲烷分別萃取苦瓜種子、果實、莖和葉等不同部位所得到的粗萃取成分中，只有乙醇萃取的果實成分，對 HepG2 及 HepG2.2.15 細胞具有較高的生長抑制作用。其中有一部份苦瓜萃取物同時對 HepG2 及 HepG2.2.15 細胞生長的抑制作用與處理的時間和苦瓜的劑量相關。研究結果顯示 HM483 及 HM17 兩個品系苦瓜的乙醇萃取物，在處理劑量高於 0.4mg/ml 的情況下，對兩株肝癌細胞就都具有明顯的生長抑制效果。本研究結果顯示，各項不同的苦瓜萃取成分，對 HepG2 及 HepG2.2.15 兩株細胞的作用並沒有明顯的差異，而因 HepG2.2.15 細胞中 HBV 的存在，也間接顯示 HBV 基因體的表現可能與 HepG2 肝癌細胞對苦瓜萃取成分的反應無關。

關鍵詞：抗病毒、抗腫瘤、苦瓜、HepG2 肝癌細胞、B 型肝炎病毒

INTRODUCTION

Hepadnavirus is a small hepatotropic DNA-containing virus that replicates through an RNA intermediate (). Huamn hepatitis B virus (HBV), a member of the hepadnavirus family, is responsible for both acute and chronic liver infection. Chronic HBV infection, which causes liver cirrhosis and hepatocellular carcinoma (HCC), is a major global public health problem (Hino et al., 2002). Over 350 millions people are chronically infected with HBV and about 1 million people die of HBV related diseases each year, although an effective vaccine has been available for 20 years, there is still no effective treatment for HBV infected individuals (Ocama et al., 2005). The clinical treatments such as interferon-α (INF-α), nucleoside analog and gene therapy strategy are of limited efficacy because of low rates of seroconversion and the rapid development of drug-resistant mutants (Shindo et al., 1999; Chiou et al., 2001; Liaw, 2002; Wolters et al., 2002). There is an urgent need for more effective antiviral therapies or alternative approaches that can completely reduce or inhibit HBV replication.

Herbal medications are currently being promoted for clinical use in preventing virus infection or cancer therapy. Many of these claims are based on anecdotes in traditional Chinese medicine. Nevertheless, it is conceivable that certain herbs or natural products could have potent pharmacological properties. *Momordica charantia*, a cucurbitaceae fruit widely consumed as a vegetable in Asia, has been reported to possess several bioactivities including antidiabetic activity, antimutagenicity, antiviral activity and cytotoxicity (Grover & Yadav, 2004). It is one of the plants that has been frequently used as medicine. MC is commonly known as bitter gourd or bitter melon in English and cultivated throughout the world for use as vegetable as well as medicine. Some of its common uses in most countries are for treatment of diabetes (Day et al., 1990; Grover et al., 2001). It was also reported to have prominent anti-virus activities on human immunodeficiency virus (HIV) and herpes simplex virus (HSV) by the MC-derived ribosome inactivating proteins, MAP30 and GAP31 (Lee-Huang et al., 1995; Bourinbaiar & Lee-Huang, 1996). Previous studies have shown that crude MC extract and its various purified fractions from some

species have anticancer activity against several malignancies such as lymphoid leukemia, lymphoma, choriocarcinoma, melanoma, breast cancer, skin tumor, prostatic cancer, squamous carcinoma of tongue and larynx, human bladder carcinomas and Hodgkin's disease (Battelli et al., 1996; Sun et al., 2001). In addition to the reported anti-tumor activity of MAP30 and GAP31, momordin, cucurbitacin and two glycoproteins, α and β momorcharins, from MC seeds have also been elucidated to inhibit guanylate cyclase of pathogenesis and replication of leukemia and other cancers (Takemoto et al., 1982; Ng et al., 1992; Rybak et al., 1994; Arazi et al., 2002). HBV is highly associated with the development of hepatocellular carcinoma. However, the bioactivities of MC on HBV and the HBV related hepatocellular carcinoma remain to be elucidated. To clarify the anti-tumor effect of MC on hepatocellular carcinoma, HBV-infected cells will simultaneously aid to reveal the possible anti-virus effect on the cells and the HBV regulatory mechanisms respond to MC. The aim of this study intends to primarily evaluate the effect of MC extracted by different parts or processes in human hepatocellular carcinoma HepG2 cells and that HBV-infected HepG2.2.15 cells.

MATERIALS AND METHODS

Preparation of MC Extracts

Fruits, stems, leaves and seeds of MC were certified and kindly donated by the District Agricultural Research and Extension Station, Council of Agriculture (Hualien, Taiwan). Different parts of the fresh M. charantia were chipped and extracted with CH_2Cl_2 and ethanol under room temperature, respectively. The CH_2Cl_2 and ethanol extracts were separately concentrated under reduced pressure to give a brownish residue. The extracts were consequently diluted with DMSO and PBS to the appropriate concentration before using for various treatments.

Cell Lines and Culture

HepG2.2.15 cells (human hepatoma cell line HepG2 stably transfected with HBV genome, kindly provided by Prof. W.L. Shih) and HepG2 cells

(human hepatocellular carcinoma, obtained from American Type Culture Collection) were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 100 ml/l fetal bovine serum (FBS), 1.5g/l sodium bicarbonate, 1.0 mmol/l sodium pyruvate, 100 IU/ml penicillin and 100 μg/ml streptomycin at 37℃ in a humidified incubator with 5% CO_2. A final concentration of 200μg/ml G418 was contained in the medium for the maintenance of HepG2.2.15 cells. Before the treatment, the cell count was adjusted to 1×10^6 /ml and cell viability to higher than 90% by trypan blue exclusion test. All cultures were free of mycoplasma.

MC Extracts Treatment and Cell Viability Assay

For MC extract treatments, 1×10^4 HepG2 and HepG2.2.15 cells were seeded onto a 96-well plate and allowed to grow for one day before treatment with different concentrations of MC extracts. After drug treatment, the cell viability was measured based on the reduction of MTT in mitochondria. A microculture tetrazolium colorimetric assay was performed to determine the cytotoxicity and cell proliferation of MC by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT, Sigma Chemical Co., St Louis, MO). After treating with MC for 0, 24 and 48 h, the media were removed and cells were washed with phosphate-buffered saline (PBS). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was then added to each well to a final concentration of 0.5 mg/ml and cells were incubated at 37℃ for 4 h. The reaction was terminated by removing the supernatant gently and the purple formazan crystals were dissolved by adding 100 μl of DMSO, following by thorough mixing for 30 min. Plates were read at 570 nm using the microplate reader (μQuant, Kcjunior, Bio-Tek Instruments, Inc. U.S.A.) according to the manufacturer's instruction. The means and standard deviations were calculated from eight replicates. Relative cellular growth was calculated by a ratio of average absorbance or counts of various treatments to that of the control ones.

Nuclear Staining with DAPI and PI

Cellular changes after treatments were evaluated by staining with PI and DAPI. HepG2 and HepG2.2.15 cells were treated with the extracts of MC for 24h before staining. PI stained cells were fixed with 80% ethanol for 30 min and incubated with a 5 μM Propidium iodide solution for 30 min in the dark. DAPI stained cells were fixed with 4% paraformaldehyde for 10 min and incubated with a 0.4 μg/ml DAPI solution for 10 min in the dark. The nuclear morphology of the cells was examined by fluorescence microscopy (Axiovert 200, Zeiss, Germany).

Statistical Analysis

For all the treatments, data were expressed as mean±SE of six independent experiments. Statistical analysis of variance followed by Student's t-test was used to assess the differences between control and cells treated with the extraction of MC.

RESULT

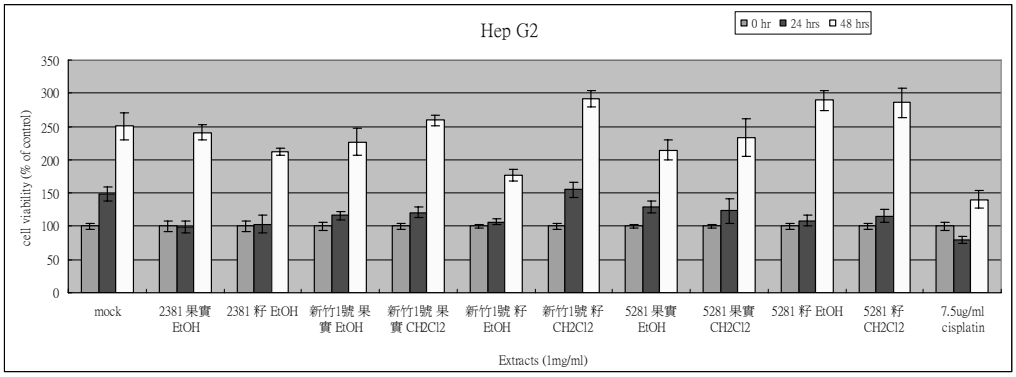
Inhibition of Cell Growth by MC

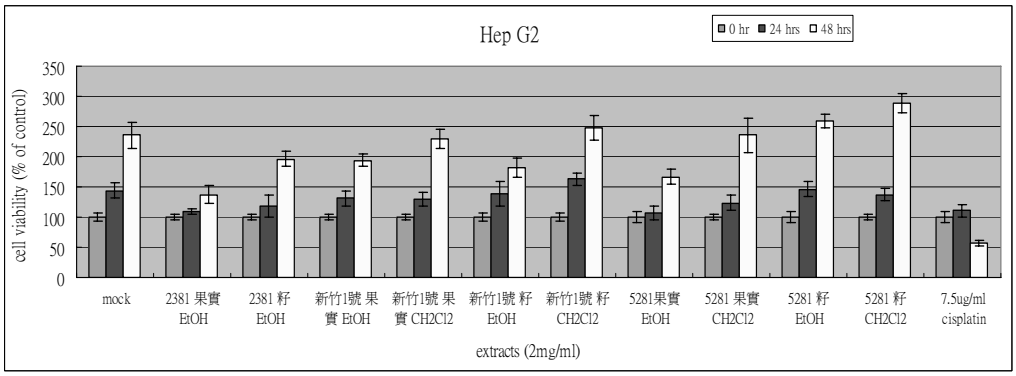
As shown in Figure 1, the growth of HepG2 and HepG2.2.15 cells were not inhibited significantly by MC after treating at a concentration of 1 or 2 mg/ml. The growth inhibition was comparatively evaluated with the control cells and the cells treated by cisplatin which is an effective chemotherapeutic agent. From the cell viability, fruit of 2381 and 5281 extracted by ethanol showed a higher inhibitory effect on cell growth at the treated concentration of 2mg/ml. In general, at the same treatment condition, the growth inhibition of MC on HepG2.2.15 cells was slightly higher than that on HepG2 cells. Among all the different species of MC, ethanol extract of HM483 and HM17 showed significant cell growth inhibition on HepG2 and HepG2.2.15 in a dose- and time-dependent manner. As shown in Figure 2, the effect of MC on HepG2 cells was slightly higher than that on HepG2.2.15 cells. The effective dose used for inhibiting the cell growth was lower than other MC extracts. In addition, the MC from different cultivated area showed significant different growth

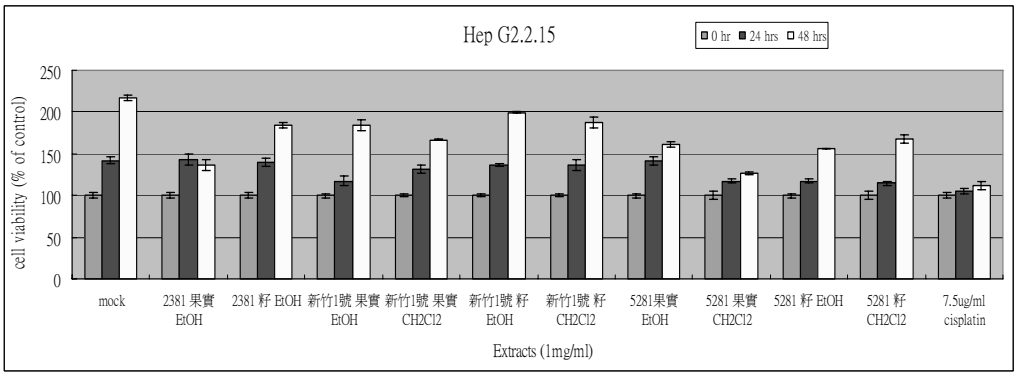
inhibition on both cells (data not shown).

Morphological Observations

After treating with HM483 and HM17, both the cells declined significantly with an increased dose. The nuclear condensation could be observed at 24 h after treatments. The difference of cellular changes between HepG2 and HepG2.2.15 was not significant.


Hep G2
0 hr
24 hrs
48 hrs
cell viability (% of control)
350
300
250
200
150
100
50
0
mock
2381 果實 EtOH
2381 籽 EtOH
新竹1號 果實 EtOH
新竹1號 果實 CH2Cl2
新竹1號 籽 EtOH
新竹1號 籽 CH2Cl2
5281 果實 EtOH
5281 果實 CH2Cl2
5281 籽 EtOH
5281 籽 CH2Cl2
7.5ug/ml cisplatin
Extracts (1mg/ml)


Hep G2
0 hr
24 hrs
48 hrs
cell viability (% of control)
350
300
250
200
150
100
50
0
mock
2381 果實 EtOH
2381 籽 EtOH
新竹1號 果實 EtOH
新竹1號 果實 CH2Cl2
新竹1號 籽 EtOH
新竹1號 籽 CH2Cl2
5281果實 EtOH
5281 果實 CH2Cl2
5281 籽 EtOH
5281 籽 CH2Cl2
7.5ug/ml cisplatin
extracts (2mg/ml)


Hep G2.2.15
0 hr
24 hrs
48 hrs
cell viability (% of control)
250
200
150
100
50
0
mock
2381 果實 EtOH
2381 籽 EtOH
新竹1號 果實 EtOH
新竹1號 果實 CH2Cl2
新竹1號 籽 EtOH
新竹1號 籽 CH2Cl2
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7.5ug/ml cisplatin
Extracts (1mg/ml)

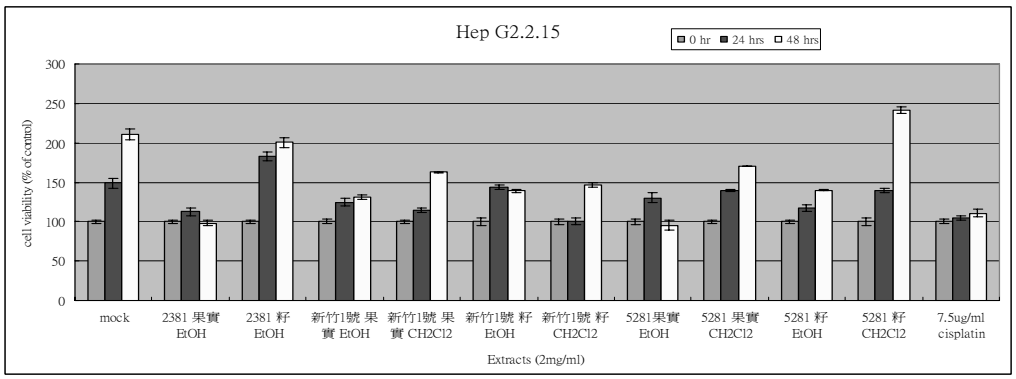

Hep G2.2.15
0 hr
24 hrs
48 hrs
cell viability (% of control)
300
250
200
150
100
50
0
mock
2381 果實 EtOH
2381 籽 EtOH
新竹1號 果實 EtOH
新竹1號 果實 CH2Cl2
新竹1號 籽 EtOH
新竹1號 籽 CH2Cl2
5281果實 EtOH
5281 果實 CH2Cl2
5281 籽 EtOH
5281 籽 CH2Cl2
7.5ug/ml cisplatin
Extracts (2mg/ml)

Figure 1.Effect of growth inhibition on HepG2 and HepG2.2.15 cells by various MC extracts. HepG2 and HepG2.2.15 cells were treated with the indicated concentrations of MC extracts for two days. MTT assays were processed as described in Materials and Methods. Data represent the cell growth inhibition rate to untreated controls (MOCK).

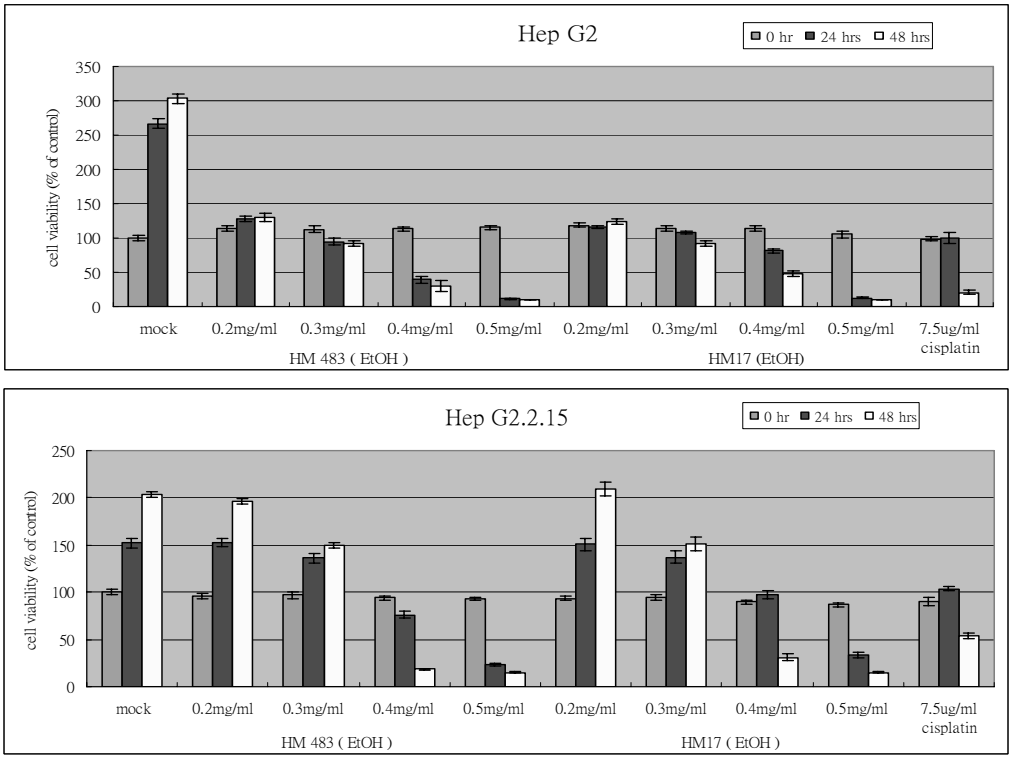


Figure 2.Effect of growth inhibition on HepG2 and HepG2.2.15 cells by ethanol extracted HM483 and HM17. HepG2 and Hep2.2.15 were treated with a gradient concentration from 0.2 to 0.5mg/ml.

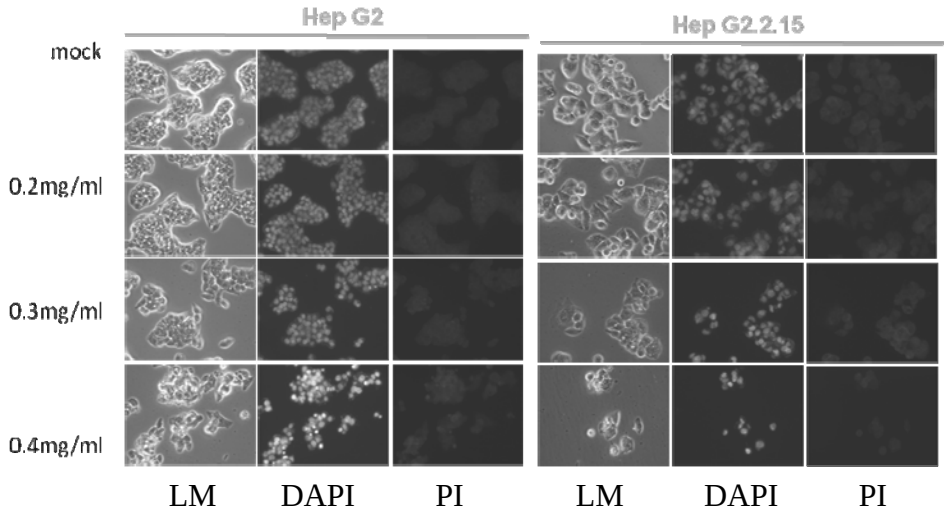


Figure 3.DAPI/PI double staining of HepG2 and HepG2.2.15 cells by treating with the ethanol extracted HM483.

DISCUSSION

Numerous attempts have been made to identify treatment for HBV infections because of the consequent diseases (Colacino, 1996; Huang et al., 2006). Among them, several components from natural products and many small molecules of herbal origin have been shown to have some anti-HBV activities in vitro. These results partly explain the beneficial effects of complementary or alternative strategy in the treatment of HBV-infected patients. According to previous report using by MAP30, a plant protein obtained from Momordica charantia, the production of HBV and expression of HBsAg and HBeAg could be inhibited by MAP30 (Fan et al., 2007). Our result from the cytotoxic analysis of MC on both HepG2 and HepG2.2.15 cells could just show a significant anti-tumor activity by an appropriate MC extract, but it didn't demonstrate any information concerning the anti-virus activity on HBV.

MAP30 and another anti-HIV protein from *Gelonium multiflorum*, GAP31, have been reported to possess dual ability to act on both DNA and RNA substrates. MAP has a novel DNA topological inactivation ability to convert viral DNA into topologically inactive forms and interrupting DNA function. The ability of MAP30 to interrupt essential topological

interconversions of viral DNA and ribosomal function of rRNA in viral-infected cells may provide novel mechanisms for its antiviral actions. MAP30 also has potent anti-tumor activity against tumor cells (Rybak et al., 1994). In addition to MAP30, extracts of MC contain many other components which are not well characterized and may contain undefined biological activities. The importance of MC in regulating cell viability is mediated by several components.

This study on dosage-cell growth relationships showed that several species of MC with appropriate extraction could inhibit the growth of HepG2 and HepG2.2.15 cells. The differences of growth inhibitory effect on HepG2 and HepG2.2.15 by specific MC extracts are not significant. With the anti-HBV data of the HepG2.2.15 cells that didn't be shown in this study, the effective MC extracts for anti-virus and anti-tumor bioactivities are different. It indicated that these bioactivities of MC extract on HepG2 cells with or without HBV were mediated by different components.

Our results in this study indicated that MC is a potent anticancer drug on both hepatocellular carcinoma cells with or without HBV infection. However, the anti-HBV activity of MC extracts on HBV-infected hepatocellular carcinoma cells and the relationship between anti-virus and anti-tumor activity in HepG2.2.15 cells remain to be investigated.

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The Anti-virus and Anti-tumor Effect of *Momordica charantia* on HepG2 and HepG2.2.15 Cells.

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ABSTRACT

To search for virus and cancer chemopreventive agents from natural resources, many phytochemicals and food additives have been screened. Bitter gourd (Momordica charantia, MC), widely known for its hypoglycemic, antiviral, antidiabetic and antitumor activities, has been used throughout the world as both food and traditional medicine. To clarify the multifunctional activities of MC with different species, the in vitro anti-virus and anti-tumor activities of crude extracts from MC were evaluated in this study by hepatocarcinoma cell lines, HepG2 cells and Hepatitis B virus (HBV)-infected HepG2 cells, HepG2.2.15 cells. MTT analysis was used to compare the cytotoxicity of cells treated with extracts of MC. Among the ethanol or CH2Cl2 extracts from MC seed, fruit, stem and leaves tested, only the ethanol extracts from the fruit inhibited the growth of HepG2 or HepG2,2,15 cells. Some extracts inhibited cell growth in both HepG2 and HepG2.2.15 cells in a time- and dose-dependent manner. Ethanol extracts from fruits of HM483 and HM17 showed significant growth inhibition on both cell lines at concentrations higher than 0.4mg/ml. There was no difference between HepG2 and HepG2.2.15 cells to all the MC extracts tested. It showed the presence of the HBV genome in the HepG2.2.15 cells did not change the response of HepG2 cells to MC extracts.

Key words：Anti-virus, Anti-tumor, *Momordica charantia*, HepG2, HBV.