

行政院國家科學委員會專題研究計畫成果報告

馬藍及芋香林投活性成分之研究

Studies on the Active Constituents of *Baphicacanthus cusia* and *Pandanus odorus*

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一、中文摘要

馬藍 (*Baphicacanthus cusia* (Nees) Bremek.) 為爵床科 (Acanthaceae) 植物，具有抗發炎、抗菌及抗癌等活性，馬藍根部之抽出物在細胞毒性方面對 p-388 具有很強的活性，因此本研究以細胞毒性當活性引導分離指標，進行成分分離因此在本研究中，便以其抗細胞毒性作為活性指標，進行化學成分之分離，共分離得到八個化合物，分別為 Lupeol (H-1), Stigmasterol 和 β -sitosterol 混合物 (H-2), Betulin (H-3), Indigo (C-1), Indirubin (C-2), 2-Benzoxazolinone (E-1) 及 2-Hydroxy-1,4-benzoxazin-3-one (E-2), 4-Hydroxy-3-methoxy benzoic acid (E-3)。

芋香林投 (*Pandanus odorus* Ridl.) 為露兜樹科 (Pandanaeae) 植物，一般當做食物或食物矯味用，另外也有降低血糖、保護肝臟、抑制腫瘤惡化等民間醫療用法。芋香林投葉部之甲醇抽出物在細胞毒性方面對 p-388 及 A-549 具有很強的活性，故本實驗以細胞毒性當活性引導分離指標，進行成分分離。本研究分離鑑定出五個化合物，分別為 β -sitosterol and stigmasterol (NH-1)、 β -sitosterol-3-O- β -D-glucoside and stigmasterol-3-O- β -D-glucoside (NH-2)、methylsuccinic anhydride (NH-3)、1,3-dimethyl-pyrrolidine-2,5-dione (NH-4)、p-hydroxycinnamic acid (NH-5)；在保肝活性試驗方面則對四氯化碳誘導之急性肝毒性

具有保護作用。

關鍵詞：馬藍、芋香林投、活性成分

Abstract

The plant *Baphicacanthus cusia* (Nees) Bremek. of Acanthaceae family has been used in traditional Chinese medicine for a long time. The plant has been used as anti-inflammatory, antibacterial and anti-carcinogenic agent. The extract of *Baphicacanthus cusia* root showed strong inhibitory effect on tumor cell lines such as p-388. Based on the cytotoxicity guided chemical fractionation, eight compounds were isolated: Lupeol (H-1), mixture of Stigmasterol and β -sitosterol (H-2), Betulin (H-3), Indigo (C-1), Indirubin (C-2), 2-Benzoxazolinone (E-1), 2-Hydroxy-1,4-benzoxazin-3-one (E-2), and 4-Hydroxy-3-methoxy benzoic acid (E-3).

Pandanus odorus Ridl. is one of the species of Pandanaeae family. It has been used as foods, flavoring or as a herb medicine effective in lowering blood sugar level, liver protection and inhibiting tumor growth. The methanolic extract of the leaves of *Pandanus odorus* Ridl. showed strong inhibitory effect on tumor cell lines such as P-388 and A-549. Based on the cytotoxicity guided chemical fractionation, five compounds were isolated: mixture of β -sitosterol and stigmasterol (NH-1)、 β -sitosterol-3-O- β -D-glucoside

and stigmasterol-3-O- β -D-glucoside (NH-2)、methylsuccinic anhydride (NH-3)、1,3-dimethyl-pyrrolidine-2,5-dione (NH-4)、*p*-hydroxycinnamic acid (NH-5). The methanol extract of *Pandanus odoratus* showed strong protective effect against carbon tetrachloride induced liver toxicity.

Keywords: *Baphicacanthus cusia*, *Pandanus odoratus* Ridl., active constituents

二、緣由與目的

馬藍 (*Baphicacanthus cusia* (Nees) Bremek.) 為爵床科 (Acanthaceae) 植物其乾燥根稱為南板藍，功能主治為清熱解毒，涼血利咽。用於溫毒發斑，舌絳紫暗，疔腮，喉痺，爛喉丹痧，大頭瘟疫，丹毒，癰腫⁽¹⁾，臨床上對於流行性感冒、肝炎⁽²⁻⁵⁾等具有顯著效果，是我國傳統中藥板藍根的基原植物之一。

茅香林投 (*Pandanus odoratus* Ridl.) 為露兜樹科 (Pandanaeae) 露兜樹屬 (*Pandanus*) 植物原產於以馬來西亞為中心的熱帶亞洲地區，其屬名由馬來文拉丁化而來，越南人稱為 *Dúa thôm*，馬來西亞及印尼則稱 *Pandan Wang*⁽⁶⁾。最早引進臺灣的時間，可追溯到越戰時期，由於具有類似芋頭之香味（但無芋頭之甜味），故有香林投，或茅香林投 (*Pandanus odoratus* Ridl.)⁽⁷⁻⁹⁾之稱。

茅香林投 (*Pandanus odoratus* Ridl.) 於東南亞地區常作為食物或食物矯味劑，近年因健康食品興起，現市售成品多為其烘乾之葉部份，供消費大眾沖泡飲用，或製成茶包、罐裝飲料等，另亦有果凍上市，一般廠商聲稱其有降血糖、保護肝臟、降尿酸、抗癌等作用。因此植物乃外來品種，市面上品名不一，臺灣北部地區有稱為七葉蘭，嘉南一帶稱為碧血素，臺灣中部則稱為桔香草、香林投、香蘭等名稱⁽⁷⁾。

本研究除分離茅香林投之成分外，並進行急性毒性試驗及保肝活性試驗。

三、結果與討論

馬藍：

Lupeol (H-1)⁽¹⁰⁻¹⁶⁾

白色粉末；m.p.: 214~215°C；IR (KBr) 3326.3 (OH), 948.1 (CH), 1458.6 (CH₂), 1381.5 (CH₃), 1041.9 (C-O) cm⁻¹；EIMS m/z 426 [M]⁺；¹H-NMR (CDCl₃) δ 0.76, 0.79, 0.83, 0.95, 0.97, 1.03 (3H each, s, 5 \times CH₃), 1.68 (3H, s, H-30), 2.38 (1H, ddd, J=10.96, 10.96, 5.46 Hz, H-19), 3.19 (1H, m, H-3), 4.57 (1H, d, J=2.4 Hz, H-29), 4.69 (1H, dd, J=2.4, 1.4 Hz, H-29).

Betulin (H-3)^(11,12,14-17)

白色粉末；m.p.: 218~220°C；IR (KBr) 3344.8 (OH), 2943.6, 1458.3, 1374.0 cm⁻¹；EIMS m/z 442 [M]⁺；¹H-NMR (CDCl₃) δ 0.76, 0.82, 0.97, 0.98, 1.02 (3H each, s, 5 \times CH₃), 1.66 (3H, s, H-30), 3.14 (1H, dd, J=10.4, 5.2 Hz, H-3), 3.33 (1H, d, J=10.7 Hz, H-28), 3.80 (1H, d, J=10.7 Hz, H-28), 4.58 (1H, br s, H-29), 4.68 (1H, br s, H-29).

Indigo (C-1)⁽¹⁸⁻²⁰⁾

藍色粉末狀；m.p.: 300°C 以上昇華；UV λ (CHCl₃) 245, 284.5, 332.5, 600 nm；IR (KBr) 3267.6 (N-H), 1628 (C=O), 1300 (C-N), 760 (苯環二取代)；EIMS m/z 262 [M]⁺(50), 205 (18), 158 (16), 130 (15), 103 (100), 75 (85).

Indirubin (C-2)⁽¹⁸⁻²¹⁾

紫紅色針晶；m.p.: 352~360°C；UV λ (CHCl₃) 242, 291, 361.5, 533.5 nm；IR (KBr) 3190.5 (N-H), 1666.6 (C=O), 1630.7 (C=C), 755.4 (苯環二取代) cm⁻¹；EIMS m/z 262 [M]⁺(8), 234 (4), 205 (4), 157 (16), 130 (6), 104 (31), 103 (27), 75 (100)；¹H-NMR (DMSO) δ 6.90 (1H, d, J=7.64 Hz, H-7), 7.01 (2H, m, Hz, H-5, 5'), 7.24 (1H, ddd, J=7.60, 7.60, 0.88 Hz, H-6), 7.39 (1H, d, J=8.12 Hz, H-7'), 7.56 (1H, ddd, J=7.68, 7.68, 1.1 Hz, H-6'), 7.64 (1H, d, J=7.44 Hz, H-4'), 8.75 (1H, d, J=7.76 Hz, H-4), 10.88 (1H, s, H-1), 10.99 (1H, s, H-1')；¹³C-NMR

(DMSO) δ 189.0 (C-3'), 171.3 (C-2), 152.8 (C-8'), 141.2 (C-8), 138.7 (C-2'), 137.5 (C-6'), 129.6 (C-6), 106.9 (C-3), 125.0 (C-4), 124.7 (C-4'), 121.7 (C-5), 121.7 (C-9), 121.7 (C-5'), 109.9 (C-7), 119.3 (C-9'), 113.7 (C-7').

2-Benzoxazolinone (BOA) (E-1) ⁽²²⁻²⁴⁾

白色針晶; m.p.: 139~141°C; IR (KBr) 3400~3500 (NH), 2600~3200 (OH), 1742.6 (C=O), 1318.1 (C-N), 1261.4 (C-O-C) cm^{-1} ; EIMS m/z 135 [M^+] (100), 91 [$M-\text{CO}_2$] ⁺ (27.14), 79 (70.05), 52 (77.01); ¹H-NMR (CDCl_3) δ 7.23~7.04 (m, 4H, H-4, H-5, H-6, H-7), 9.77 (br. s, 1H, NH); ¹³C-NMR (Acetone- d_6) δ 153.8 (C-2), 109.4 (C-4), 123.5 (C-5), 121.8 (C-6), 109.2 (C-7), 143.7 (C-8), 130.2 (C-9).

2-Hydroxy-1,4-benzoxazin-3-one (HBOA) (E-2) ^(25,26)

黃色針晶; m.p.: 187~181°C; IR (KBr) 3300~2800 (OH 或 NH), 1679.5 (C=O) cm^{-1} ; EIMS m/z 165 [M^+] (26.74), 136 (100), 108 (26.39), 80 (53.82), 63 (21.01), 52 (27.08); ¹H-NMR (DMSO) δ 5.45 (1H, br. s, H-2), 6.87~6.97 (1H, m, 4H, H-5, H-6, H-7, H-8), 7.95 (1H, br. s, 2-OH), 10.78 (1H, br. s, H-4); ¹³C-NMR (CDCl_3) δ 162.8 (C-3), 140.9 (C-9), 126.9 (C-10), 123.3 (C-6), 122.7 (C-7), 117.6 (C-8), 115.8 (C-5), 90.5 (C-2).

4-Hydroxy-3-methoxy benzoic acid (vanillic acid) (E-3) ⁽²⁷⁻³¹⁾

白色粉末; m.p.: 187~181°C; IR (KBr) 3486.7 (OH), 2990.7 (CH), 1683.9 (C=O), 1242.4 (C-O-) cm^{-1} ; EIMS m/z 168 [M^+] (100.00), 153 [$M-\text{Me}$] ⁺ (75.31), 151 (19.14), 125 (20.68), 97 (44.44), 79 (15.35), 51 (29.94); ¹H-NMR (Acetone- d_6) δ 3.90 (s, 3H, 3-OCH₃), 6.91 (1H, d, $J=8.08$ Hz, H-5), 7.56 (1H, d, 1.94 Hz, H-2), 7.59 (1H, dd, 8.08, 1.96 Hz, H-6); ¹³C-NMR (Acetone- d_6) δ 166.3 (C=O), 150.9 (C-4), 146.9 (C-3),

123.7 (C-6), 121.8 (C-1), 114.4 (C-5), 112.3 (C-2), 55.2 (-OCH₃).

本實驗由馬藍根部抽出物鑑定出八個化合物，其中 lupeol (H-1)、phytosterol (stigmasterol, β -sitosterol 的混合物) (H-2)、betulin (H-3)、indigo (C-1)、indirubin (C-2) 等為已知之主成分，而 2-benzoxazolinone (E-1)、2-Hydroxy-1,4-benzoxazin-3-one (E-2) 則為本屬植物中首次分離得到之成分。細胞毒性引導分離結果，對所偵測細胞並無明顯抑制作用。

芋香林投：

Methylsuccinic anhydride (NH-3) ^(32,33)

白色半透明結晶; m.p.: 113.2~115.1°C; IR (KBr) 1696 (-OH) cm^{-1} ; EI-MS m/z 114 (M^+ , 28.31); ¹H-NMR (CDCl_3) δ 1.30 (3H, d, $J=7.14$ Hz, -CH₃), 2.56 (1H, dd, $J_1=4.88$, $J_2=16.98$ Hz, H4 α), 2.74 (1H, dd, $J_1=8.62$, $J_2=16.98$ Hz, H4 β), 2.93 (1H, m, H3); ¹³C-NMR (CDCl_3) δ 16.7 (-CH₃), 35.5 (-CH, C-3), 37.2 (-CH₂, C-4), 177.9 (C=O, C-2), 181.4 (C=O, C-5)

1,3-Dimethyl-pyrrolidine-2,5-dione (NH-4) ⁽³⁴⁾

白色片狀結晶, m.p.: 103~106°C; IR (KBr) 1697 (C=O), 3326 (-OH) cm^{-1} ; EI-MS m/z 127 (M^+ , 6.60); ¹H-NMR (CDCl_3) δ 1.22 (3H, d, $J=6.96$ Hz, CH₃), 2.44 (1H, dd, $J_1=5.56$, $J_2=16.52$ Hz, H-4 α), 2.78 (1H, dd, $J_1=8.08$, $J_2=16.26$ Hz, H-4 β), 2.95~2.82 (1H, m, H3), 3.68 (s, N-CH₃); ¹³C-NMR (CDCl_3) δ 16.9 (-CH₃), 35.5 (-CH), 37.2 (-CH₂), 252.1 (N-CH₃), 175.7 (C=O), 177.1 (C=O)

p-Hydroxycinnamic acid (NH-5) ⁽³⁴⁻³⁷⁾

淡黃白色結晶; m.p. 188.4~194.8°C; IR (KBr) 3388 (-OH), 1674 (C=O) cm^{-1} ; EI-MS m/z 167 (M^+ , 100); ¹H-NMR (CDCl_3) δ 6.26 (1H, d, $J=15.92$ Hz, CH-C-ph), 6.77 (2H, d,

$J= 8.58\text{Hz}$, H3, H5), 7.46 (1H, d, $J= 15.98\text{Hz}$, C= CH-ph), 7.49 (2H, d, $J= 8.52\text{Hz}$, H2, H6); ^{13}C - NMR (CDCl_3) δ 115.7 (C-3, C-5, C-8), 125.5 (C-1), 130.3 (C-2, C-6), 159.7 (C-4), 144.3 (C-7), 168.0 (C-9)

自芋香林投葉部抽提物中鑑定出五個化合物，其中 β - sitosterol and stigmasterol (NH-1), β -sitosterol-3-O- β -D-glucoside stigmasterol-3-O- β -D-glucoside (NH-2), Methylsuccinic anhydride (NH-3), 1,3-dimethyl-pyrrolidine-2,5-dione (NH-4), *p*-hydroxycinnamic acid (NH-5). 為本屬植物中首次分離得到之成分。

芋香林投之鼯鼠急性毒性試驗，結果顯示其半數致死劑量 LD_{50} ，在給予 10g/kg 、 20g/kg 未見到鼯鼠死亡情形，且其活動力與投藥前無特殊差異，可知其急性毒性很低，一般當作食品使用應無安全顧慮。對四氯化碳(carbon tetrachloride)所誘發之 sGOT、sGPT 值急速上升，均呈有意義的抑制作用($p < 0.01$)，據統計學(ANOVA 及 N-K test)分析發現；以 0.5g/kg 效果最好，並且在三組劑量中以 0.5g/kg 、 1g/kg 保肝效果優於對照組，顯示芋香林投對於肝臟發炎有預防之效果。

四、計畫成果自評

馬藍被當成板藍根使用，且稱其為抗菌要藥，對馬藍根部做成分單離及鑑定後，我們發現從分離出的成分與文獻考查知 indirubin 具有抗白血病作用，故可推論此成分與其抗菌藥理活性有相當關係，然所分離得到之化合物對所測試之腫瘤細胞，並未具有明顯的抑制作用，是否有其它有效成分或對其它腫瘤細胞有抑制作用，仍待我們繼續研究及探討印證之。

芋香林投粗抽物之細胞毒性試驗結果，顯示尚有繼續分離研究的空間，而鼯鼠急性毒性試驗則可知其急性毒性很低，作為食品使用時，無安全顧慮。而當劑量

0.5g/kg 時，對四氯化碳誘導之肝發炎具有保護肝臟的效果。根據以上結果之討論，顯示芋香林頭作為健康食品應可被接受而無害，而其他效用仍可繼續探討研究。

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