美和學校財團法人美和科技大學

103年度教師產學合作計畫

結案報告書

計畫名稱：大益牛樟芝活性指標成分之分離純化與鑑定

計畫編號：103-

計畫期間：102/11/01~103/07/31

計畫主持人：林昀生

共同主持人：吳裕仁、鄭智交、黃漢翔

研究助理：陳泓吉

經費總額：　200,000　　　　　　　元

經費來源：大益生物科技有限公司

目錄

一、計畫成果摘要：

牛樟芝(*Antrodia cinnamomea*)為擔子菌門擬層孔菌科的藥用性真菌類，用於養生保健，只生長在台灣特有種高海拔的常綠闊葉大喬木的牛樟樹（*Cinnamomum kanehirae*）上，在傳統療法，牛樟芝被喻為是一種「補肝良藥」。近年來許多營養學家及倡導保健之人士推廣健康食療，使得保健食品開始被重視，也才有今日台灣食品業界及中、西醫藥界對保健產品的開發及推廣，並使牛樟芝得到國內健康食品認證。其亦為護肝功能、免疫調節等健康食品開發的寶貴資源。由於該公司皆以段木栽培方式培育牛樟芝子實體，本計畫委託以科學驗證方式，萃取其牛樟芝其內含成分，透過分析實驗，分離純化出其內含指標成分，以達到牛樟芝其有效成分之利用。

本實驗依據102年2月5日經濟部標準檢驗局國家標準審查委員會審定通過牛樟芝的國家標準規範，對於大益生技公司所培育的牛樟芝子實體進行萃取與分析其內含指標成分，發現該公司的牛樟芝子實體經萃取後，內含該規範之八大指標成分物質，包含以下化合物:

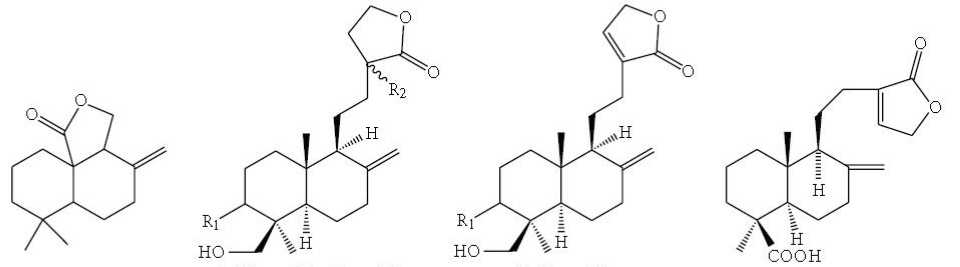
1. Antcin A
2. Antcin B
3. Antcin C
4. Antcin K
5. Antcin H
6. 1,4-di-methoxy-2,3-methylenedioxy-5-methylbenzene
7. dehydrosulfurenic acid
8. dehydroeburicoic acid

因此，該實驗成果證明大益生技所培育的牛樟芝子實體具有以上之成分物質，本實驗以供未來申請健康食品認證之有效成分參考。

二、過去文獻

(從 *Antrodia camphorate* 萃取分離純化出的化合物總覽與其化合物之生理活性測試）

1. Review of Pharmacological Effects of *Antrodia camphorate* And Its Bioactive Compounds



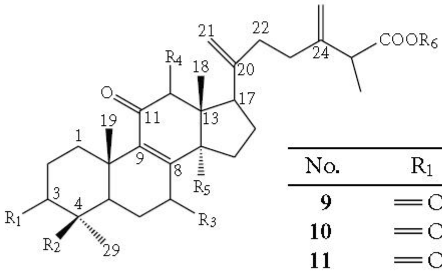
1.

2. $R_1 = H_2, R_2 = H$
3. $R_1 = OH, R_2 = H$
4. $R_1 = OH, R_2 = H$

5. $R_1 = H_2$
6. $R_1 = OH$
7. $R_1 = OH$

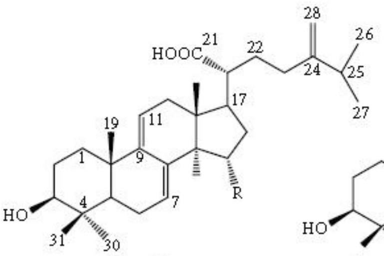
8.

(a)

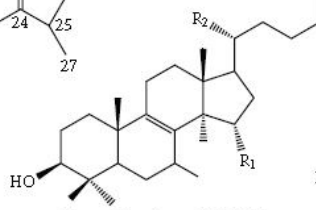


No.	R_1	R_2	R_3	R_4	R_5	R_6
9	=O	H	H_2	H_2	H	H
10	=O	H	=O	H_2	H	H
11	=O	H	β-OH	H_2	H	H
12	=O	H	=O	H_2	OH	H
13	=O	H	H_2	H_2	H	$H\Delta^{14}$
14	=O	H	β-OH	H_2	–	$H\Delta^{14}$
15	=O	H	α-OAc	H_2	–	H
16	α-OH	H	=O	α-OH	H	H
17	α-OH	H	=O	H_2	H	H
18	α-OH	β-OH	β-OH	H_2	H	H
19	=O	H	H_2	H_2	H	CH_3
20	=O	H	=O	H_2	H	CH_3
21	=O	H	=O	H_2	H	C_2H_5
22	=O	H	α-OAc	H_2	H	CH_3
23	α-OH	H	=O	α-OH	H	CH_3
24	α-OH	H	=O	α-OH	H	C_2H_5

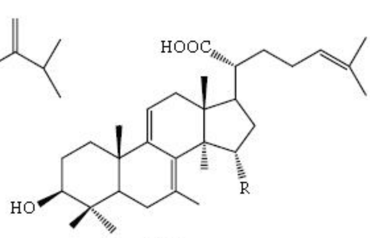
(b)



25. R = H
26. R = OH
27. R = OAc

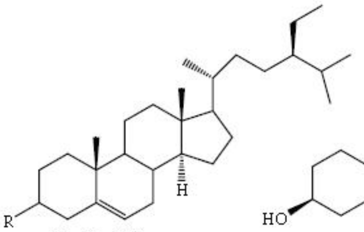


28. $R_1 = H_2$, $R_2 = COOH$
29. $R_1 = OH$, $R_2 = COOH$
30. $R_1 = OAc$, $R_2 = COOH$
31. $R_1 = H_2$, $R_2 = CH_3$

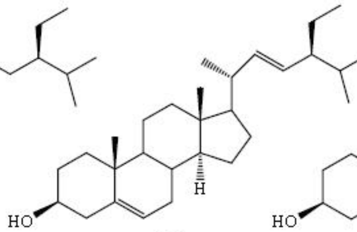


32. R = OH
33. R = H

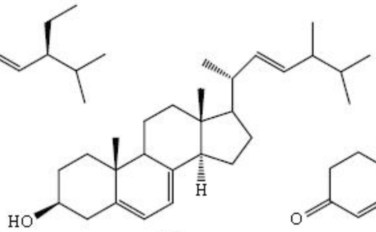
(c)



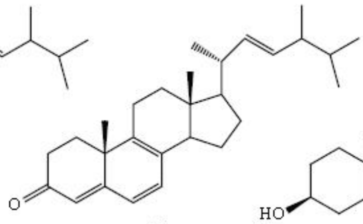
34. R = H
35. R = (═O)



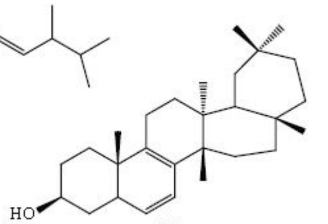
36.



37.

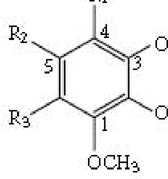


38.

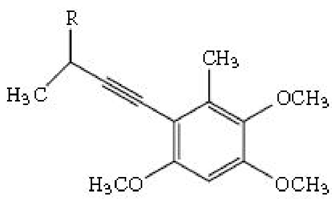


39.

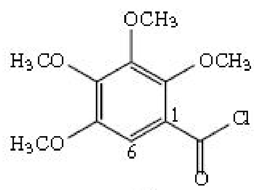
(d)



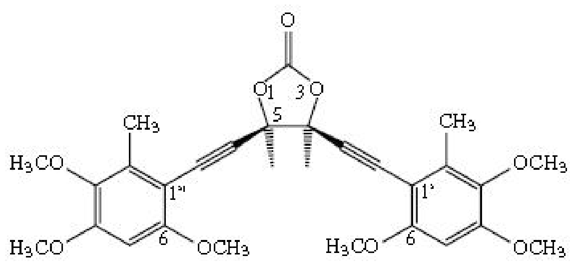
40. $R_1 = OCH_3$, $R_2 = CH_3$, $R_3 = H$
41. $R_1 = OCH_3$, $R_2 = CO_2$, $R_3 = H$
42. $R_1 = COOH$, $R_2 = H$, $R_3 = OCH_3$



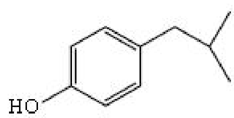
43. R = (═CH_2)
44. R = (═O)



45.

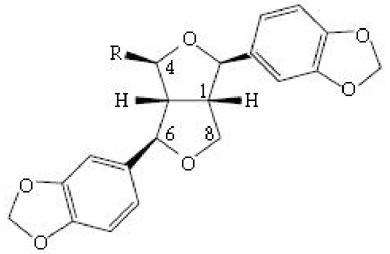


46.

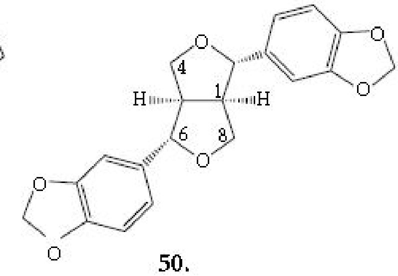


47.

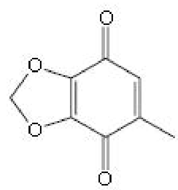
(e)



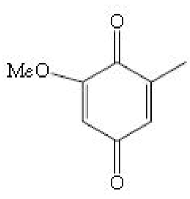
48. R = H
49. R = OH



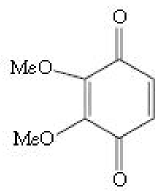
50.



51.

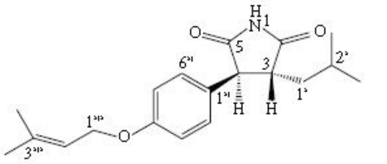


52.

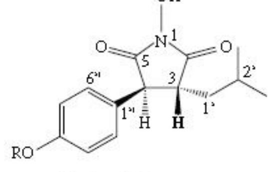


53.

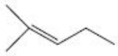
(f)

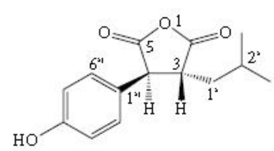


54.

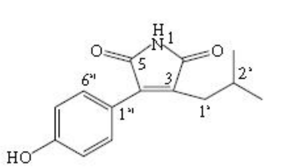


55. R = H

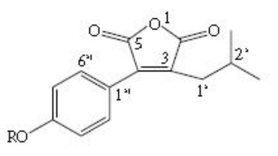
56. R = 



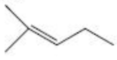
57.

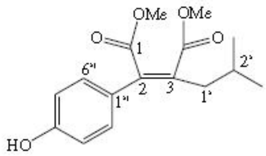


58.

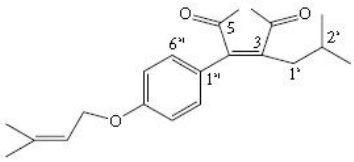


59. R = H

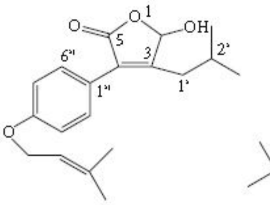
60. R = 



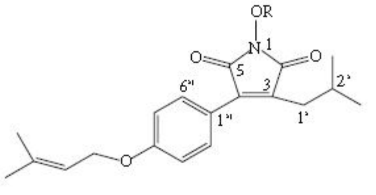
61.



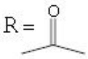
62.

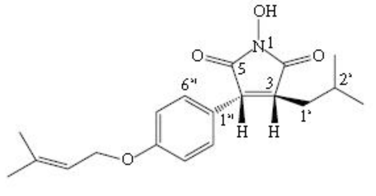


63.

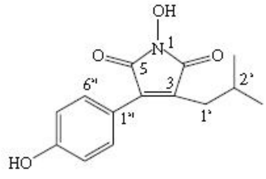


64. R = H

65. R = 

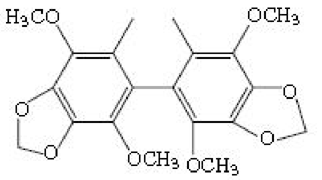


66.

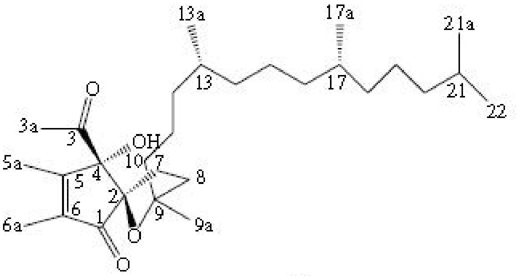


67.

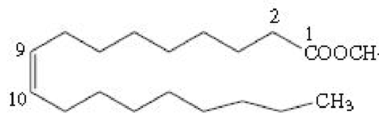
(g)



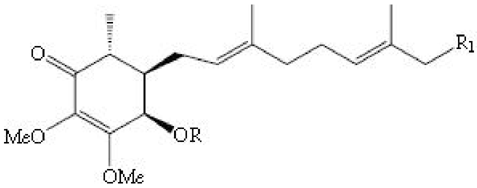
68.



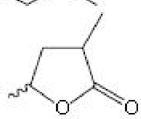
69.

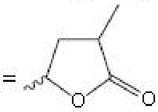


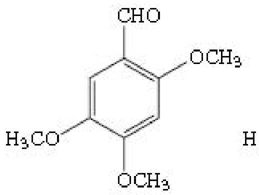
70.



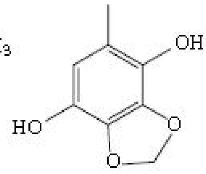
71. R = H, R_1 = 

75. R = H, R_1 = 

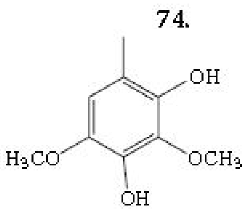
76. R = Ac, R_1 = 



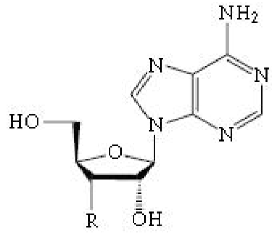
74.



77.



78.



72. R = OH

73. R = H

(h)

SOURCE: **F:Fruiting bodies (子實體)**
M: Mycelium (菌絲體)
B: Culture broth.(液態培養)

Table 1: Chemical constituents and their reported activities of *A. camphorata*.

No.	Compound name	Source	Biological activity	Ref.
	Terpenoids			
1	Antrocin	F		[19]
2	19-Hydroxylabda-8(17)-en-16,15-olide	F	*In vitro* neuroprotective	[28]
3	3β,19-Dihydroxylabda-8(17),11E-dien-16,15-olide	F	*In vitro* neuroprotective	[28]
4	13-*epi*-3β,19-Dihydroxylabda-8(17),11E-dien-16,15-olide	F	*In vitro* neuroprotective	[28]
5	19-Hydroxylabda-8(17),13-dien-16,15-olide	F	*In vitro* neuroprotective	[28]
6	14-Deoxy-11,12-didehydroandrographolide	F	*In vitro* neuroprotective	[28]
7	14-Deoxyandrographolide	F		[28]
8	Pinusolidic acid	F		[28]
9	Antcin A	F	*In vitro* anti-inflammatory, anti-insecticidal and cytotoxic	[30, 64, 65]
10	Antcin B (Zhankuic acid A)	F	*In vitro* anti-inflammatory, anti-insecticidal and cytotoxic	[18, 30, 64–66]
11	Antcin C	F	*In vitro* anti-inflammatory and cytotoxic	[65, 67]
12	Antcin D (Zhankuic acid F)	F		[23]
13	Antcin E	F		[21]
14	Antcin F	F		[21]
15	Antcin G	F		[21]
16	Antcin H (Zhankuic acid C)	F	*In vitro* anti-inflammatory, anti-insecticidal and cytotoxic	[18, 64–66]
17	Antcin I (Zhankuic acid B)	F	*In vitro* anti-inflammatory	[66]
18	Antcin K	F	*In vitro* anti-inflammatory	[66]
19	Methyl antcinate A	F		[25]
20	Methyl antcinate B	F	*In vitro* anti-insecticidal and cytotoxic	[64, 65]
21	Zhankuic acid D	F		[25]
22	Methyl antcinate G	F		[21]
23	Methyl antcinate H	F		[21]
24	Zhankuic acid E	F		
25	Dehydroeburicoic acid	F	*In vitro* anti-inflammatory, anti-insecticidal	[64, 65, 67]
26	Dehydrosulphurenic acid	F	*In vitro* anti-insecticidal and cytotoxic	[64, 65]
27	15α-Acetyl-dehydrosulphurenic acid	F	*In vitro* anti-insecticidal and cytotoxic	[64, 65]
28	Eburicoic acid	F	*In vitro* anti-insecticidal and cytotoxic	[64, 65]
29	Sulphurenic acid	F	*In vitro* anti-insecticidal and cytotoxic	[64, 65]
30	Versisponic acid D	F		
31	Eburicol (24-methylenedihydrolanosterol)	F	*In vitro* anti-inflammatory	[30]
32	3β, 15α-Dihydroxy lanosta-7,9(11),24-triene-21-oic acid	F	*In vitro* anti-insecticidal and cytotoxic	[64, 65]
33	3β-Hydroxy lanosta-	F		[17]
34	β-Sitosterol	F		[28]

Table 1: Continued.

No.	Compound name	Source	Biological activity	Ref.
35	β-Sitostenone	F		[28]
36	Stigmasterol	F		[28]
37	Ergosterol	F		[28]
38	Ergosta-4,6,8(14)22-tetraen-3-on3	F		[30]
39	*epi*-Friedelinol	F		[30]
	Benzenoids			
40	1,4-Dimethoxy-2,3-methylenedioxy-5-methylbenzene	F	*In vitro* cytotoxic	[68]
41	1,4-Dimethoxy-2,3-methylenedioxy-5-benzoate	F		[24]
42	1,6-Dimethoxy-2,3-methylenedioxy-4-benzoic acid	F		[24]
43	Antrocamphin A	F	*In vitro* anti-inflammatory	[30]
44	Antrocamphin B	F		[30]
45	2,3,4,5-Tetramethoxybenzoyl chloride	F		[30]
46	Antrodioxolanone	F		[30]
47	Isobutylphenol			[34]
	Lignans			
48	(+) Sesamin	F		[20]
49	4-Hydroxy sesamin	F		[20]
50	(−) Sesamin	F		[20]
	Benzoquinone derivatives			
51	5-Methyl-benzo(1,3)-dioxole-4,7-dione	M		[20]
52	2-Methoxy-5-methyl(1,4)benzoquinone	M	*In vitro* anti-oxidant	[20]
53	2,3-Dimethoxy-5-methyl(1,4)benzoquinone	M	*In vitro* anti-inflammatory	[20, 30]
	Succinic and Maleic derivatives			
54	*trans*-3-Isobutyl-4-[4-(3-methyl-2-butenyloxy)phenyl]pyrrolidine-2,5-dione	F	*In vitro* anti-inflammatory	[33]
55	*trans*-1-Hydroxy-3-(4-hydoxyphenyl)-4-isobutylpyrrolidine-2,5-dione	F	*In vitro* anti-inflammatory	[33]
56	*3R**,*4S**-1-Hydroxy-3-isobutyl-4-[4-(3-methyl-2-butenyloxy)phenyl]pyrrolidine-2,5-dione (antrodin D or Camphorataimide E)	F, M	*In vitro* anti-inflammatory, anti-HBV and anti-HCV	[29, 33, 69]
57	*cis*-3-(4-Hydroxyphenyl)-4-isobutyldihydrofuran-2,5-dione	F	*In vitro* anti-inflammatory	[33]
58	3-(4-Hydroxyphenyl)-4-isobutyl-1*H*-pyrrole-2,5-dione	F	*In vitro* anti-inflammatory	[33]
59	3-(4-Hydroxyphenyl)-4-isobutylfuran-2,5-dione (Antrocinnamomin C)	F	*In vitro* anti-inflammatory	[33, 35]
60	3-Isobutyl-4-[4-(3-methyl-2-butenyloxy)phenyl]furan-2,5-dione (antrodin A or Camphorataanhydride A)	M	*In vitro* anti-HBV and anti-HCV	[29, 69]
61	Dimethyl 2-(4-hydroxyphenyl)-3-isobutylmaleate	F	*In vitro* anti-inflammatory	[33]
62	3-Isobutyl-4-[4-(3-methyl-2-butenyloxy)phenyl]-1*H*-pyrrole-2,5-dione (Antrodin B or Camphorataimide B)	M, B	*In vitro* anti-inflammatory, anti-HBV and anti-HCV	[26, 29, 69]

No.	Compound name	Source	Biological activity	Ref.
63	Antrocinnamomin D	M		[35]
64	3-Isobutyl-4-[4-(3-methyl-2-nyloxy)phenyl]-1*H*-pyrrol-1-ol-2,5-dione (antrodin C or) Camphorataimide C)	M	*In vitro* anti-inflammatory, anti-HBV and anti-HCV	[28, 31, 70]
65	Antrocinnamomins A	M	*In vitro* anti-inflammatory	[37]
66	3*R**,4*R**-1-Hydroxy-3-isobutyl-4-[4-(3-methyl-2-butenyloxy)phenyl]pyrrolidine-2,5-dione (Antrodin E or Camphorataimide D)	M	*In vitro* anti-HBV and anti-HCV	[31, 70]
67	Antrocinnamomins B	M	*In vitro* anti-inflammatory	[37]
	Miscellaneous compounds			
68	2,2′,5,5′-Tetramethoxy-3,4,3′,4′-bi-methylenedioxy-6,6′-dimethylbiphenyl	F	*In vitro* anti-HBV	[71]
69	α-Tocospiro B	F		[30]
70	Methyl oleate	F		[20]
71	Antroquinonol	M, F	*In vitro* cytotoxic, anti-inflammatory, anti-HBV	[12, 32, 72]
72	Adenosine	M	Prevention of PC 12 cells apoptosis	[37]
73	Cordycepin	M		[37]
74	2,4,5-trimethoxybenzaldehyde	M	Prevention of PC 12 cells apoptosis	[31]
75	Antroquinonol B	M	*In vitro* anti-inflammatory	[36]
76	4-acetyl-antroquinonol B	M	*In vitro* anti-inflammatory	[36]
77	2,3-(methylenedioxy)-6-methylbenzene-1,4-diol	M	*In vitro* anti-inflammatory	[36]
78	2,4-dimethoxy-6-methylbenzene-1,3-diol	M	*In vitro* anti-inflammatory	[36]

F: Fruiting bodies; M: Mycelium; B: Culture broth.

三、研究方法、進行步驟

一、經由段木栽培之牛樟芝子實體以 95% 乙醇萃取三次，萃取液分別濃縮後則可得該子實體之粗萃取物(crude extract)。

二、活性成分之分離：

將各粗萃取物先以乙酸乙酯與水進行液液分配(partition)，則可得乙酸乙酯層及水層二部分，經濃縮後分別以下述方法作進一步的分離：

(1) 乙酸乙酯層的分離 ： 此層先以矽膠管柱進行分離，利用溶劑之極性高低以梯度沖堤的方式配合矽膠及分子大小篩Sephadex LH-20 等管柱層析交替進行分離，再配合高壓液相層析儀(HPLC)，以正相或逆相系填充物 (如 RP-18) 進行分離，以便得到純化合物。

(2) 水層的分離 ： 此層均屬於高極性化合物，先以正丁醇(n-BuOH)與水進行液液分配(partition)，將正丁醇層濃縮。再以 Diaion HP-20 管柱分離，沖提 溶媒依次為 H_2O、MeOH：H_2O (2：8)~MeOH：H_2O (1：1) 及 MeOH。

所沖堤出之 fraction 再利用 Sephadex LH-20 及逆相系統填充物進行分離純化，並配合高壓液相層析儀(HPLC)，以逆相系統填充物 (如 RP-18) 進行分離，以便得到純化合物。

三. 活性成份之構造鑑定：

利用核磁共振儀(Nuclear Magnetic Resonance, NMR)測定化合物之氫譜、碳譜、DEPT以及2D圖譜如1H-1H COSY、HMQC、HMBC及NOESY等，質譜儀(mass spectrometry, MS)測量化合物的分子量或分子式，紅外線光譜儀(infrared spectrum, IR)、紫外線光譜儀(Ultraviolet-Visible Spectrophotometer, IR)及旋光（Polarimeter）分別測定分子的各種物理數據。

有機溶劑（如酒精）萃取活性分析活性分析具活性之Fraction 分離純化活性純天然化合物 化學結構解析紅外、紫外光譜、質譜、核磁共振必要時做化學反應或單晶培養再以X-ray 繞射分析確定分子構造。

四. 經由 HPLC、LC-MS-MS 定性、定量牛樟芝之內含成分之含量比率。

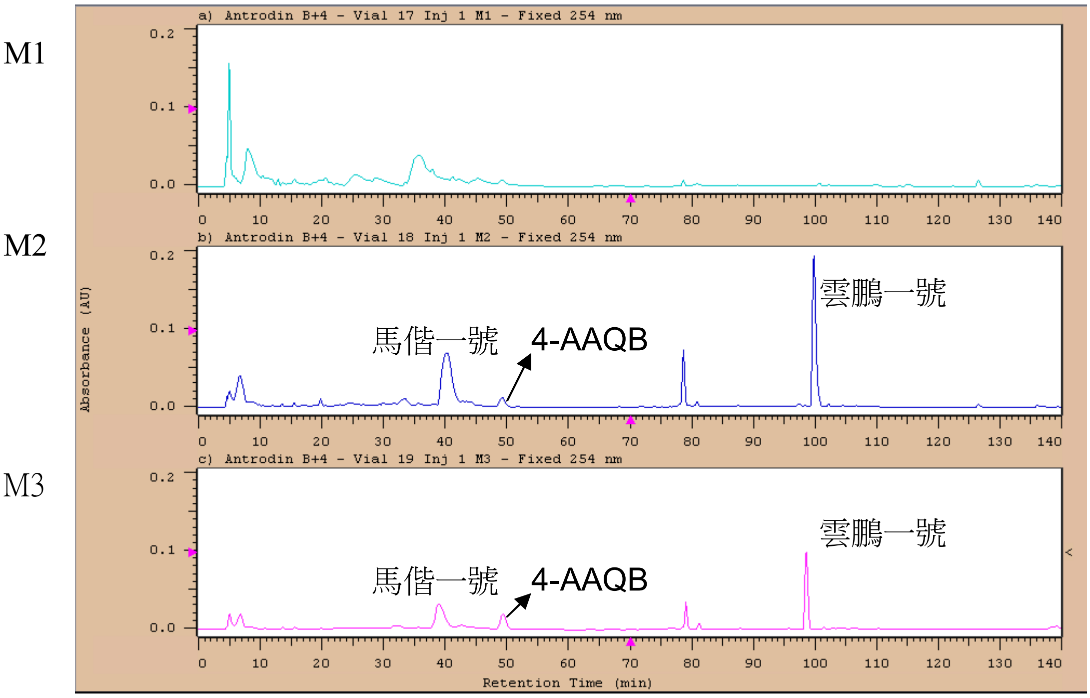
四、結果報告:

批次一:

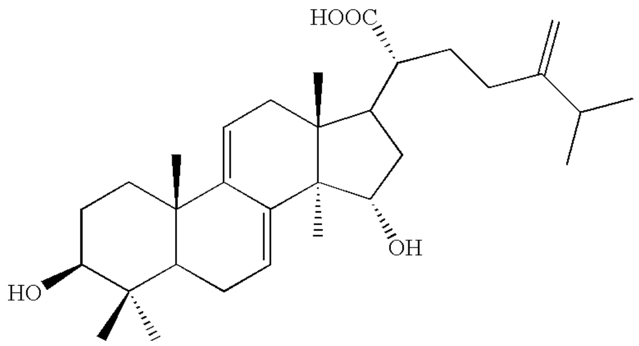
1. 送件日期 : 2013/11/28

2. 樣品 :

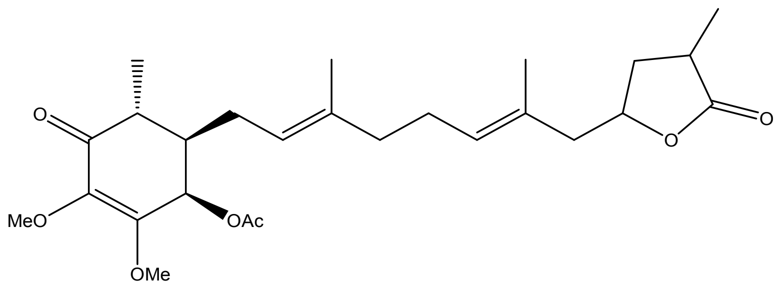
M1: 牛樟木子實體 ;
M2: 國內牛樟木類子實體 ;
M3: 進口木頭類子實體



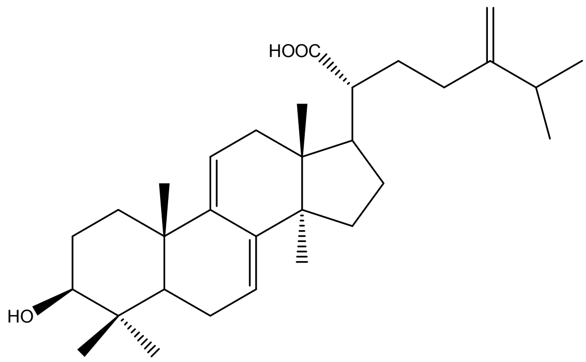
(1)



Dehydrosulfurenic acid (馬偕一號) (**26**)

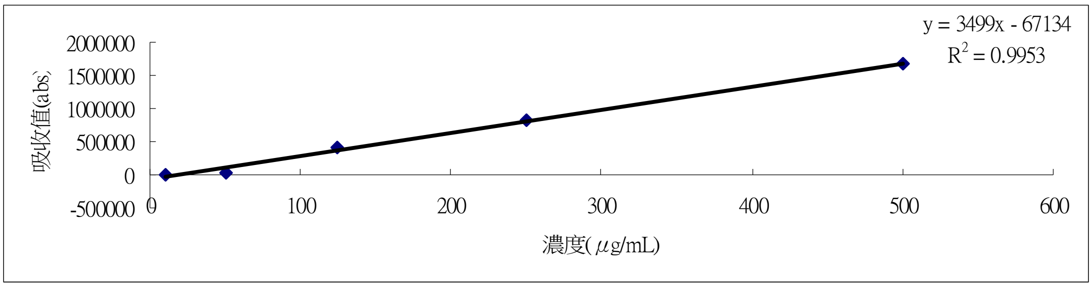


4-AAQB(4-acetyl antroquinonol B) (**76**)

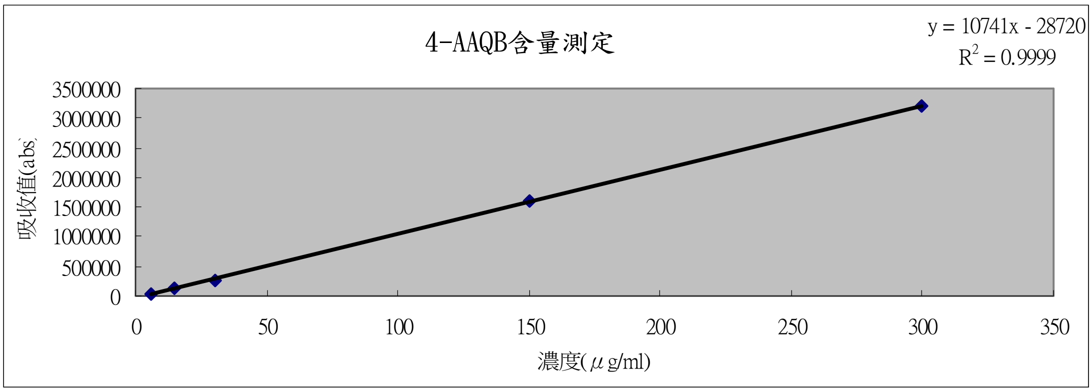


Dehydroeburicoic acid(雲鵬一號) (**25**)

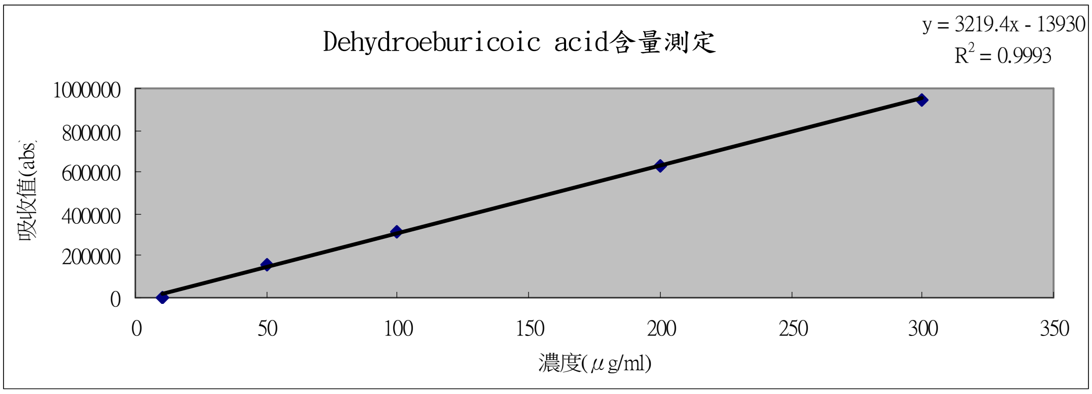
Dehydrosulphurenic acid (馬偕一號) (**26**)



4-AAQB(4-acetyl antroquinonol B) (**76**)



Dehydroeburicoic acid(雲鵬一號) (**25**)



定量

M2

Dehydroeburicoic acid(雲鵬一號) 14.83 mg/g

4-AAQB(4-acetyl antroquinonol B) 0.5 mg/g

Dehydrosulphurenic acid (馬偕一號) 14.31 mg/g

M3

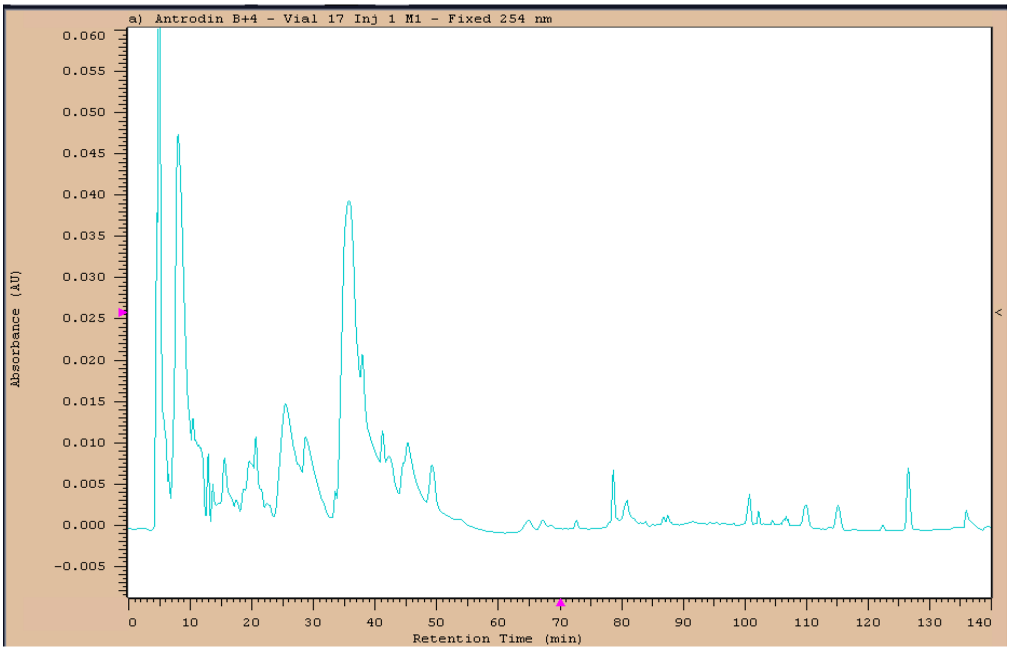
Dehydroeburicoic acid(雲鵬一號) 6.07mg/g

4-AAQB(4-acetyl antroquinonol B) 0.83mg/g

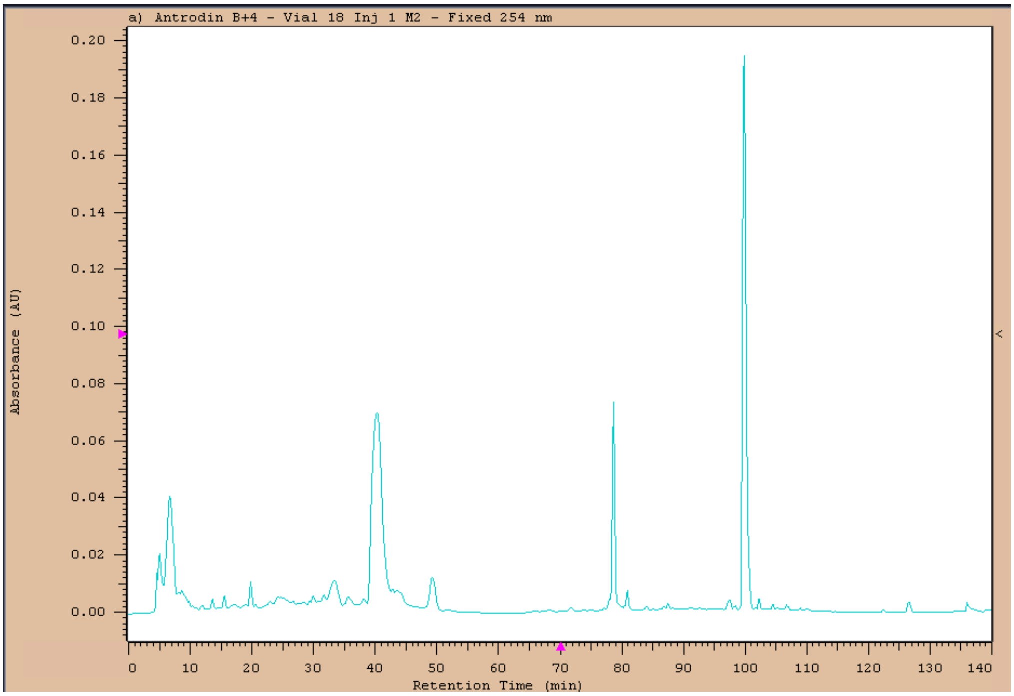
Dehydrosulphurenic acid (馬偕一號) 6.14 mg/g

以下為單獨放大指紋圖譜

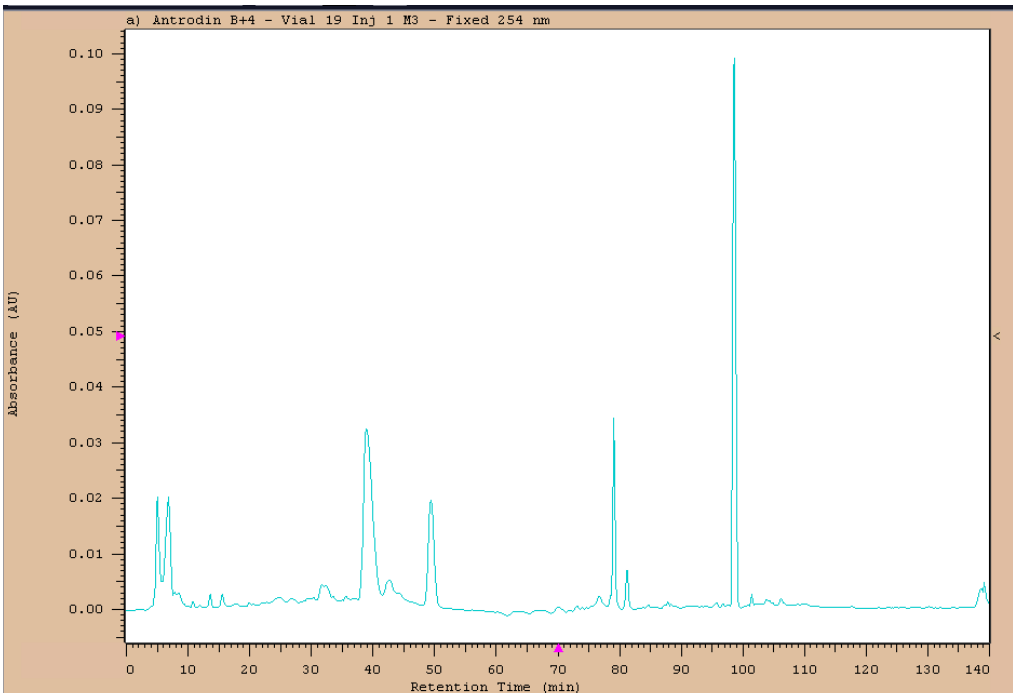
M1



M2


a) Antrodin B+4 - Vial 18 Inj 1 M2 - Fixed 254 nm
Absorbance (AU)
Retention Time (min)

M3

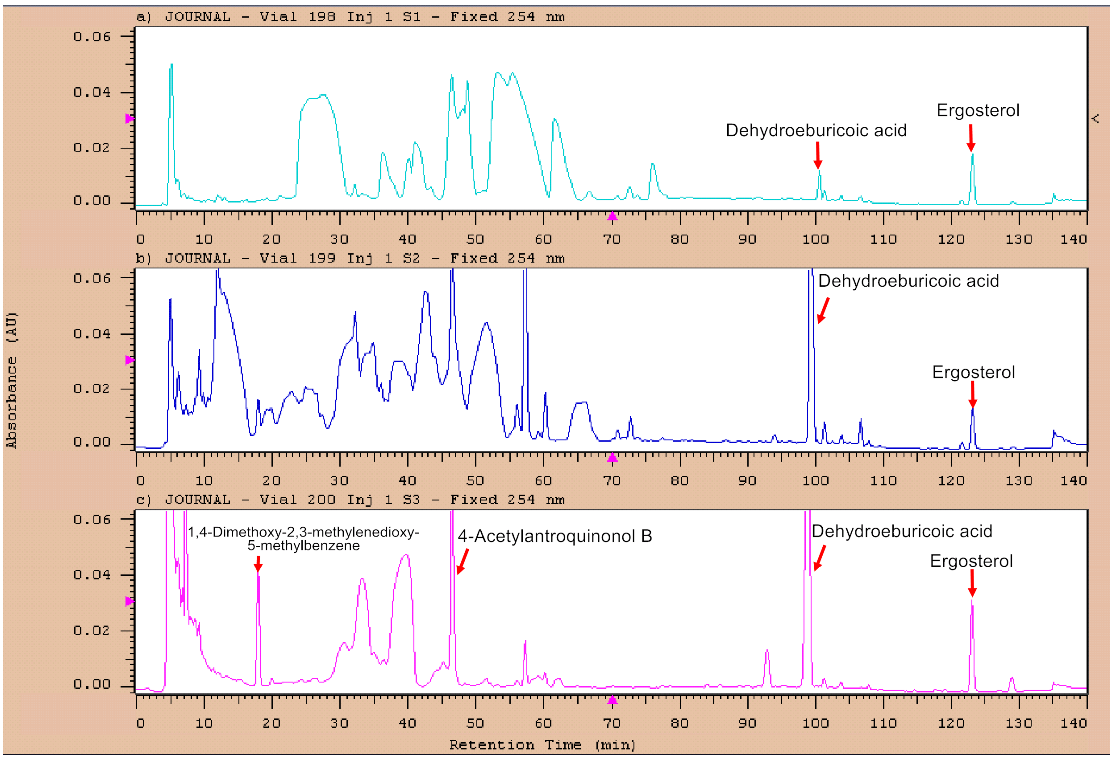

a) Antrodin B+4 - Vial 19 Inj 1 M3 - Fixed 254 nm
Absorbance (AU)
Retention Time (min)

批次二:

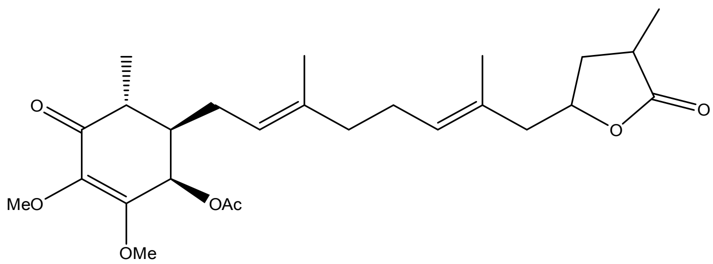
1. 送件日期 ： 2014/02/18

2. 樣品 ：

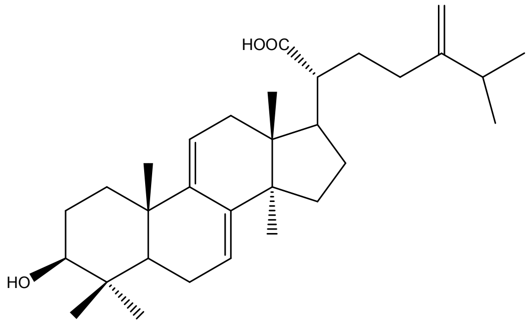
S1(牛樟木子實體)
S2(牛樟木上的類子實體)
S3(進口木頭上的類子實體)



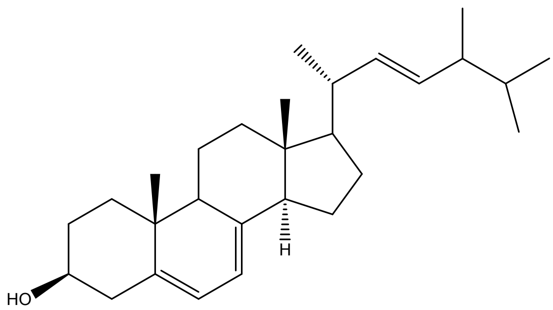
檢測日期	sample	4-AAQB (**76**) 含量 (mg/g)	Dehydroeburicoic acid(**25**)含量 (mg/g)	Ergosterol (**37**)含量 (mg/g)	1,4-Dimethoxy-2,3-methylenedioxy-5-methylbenzene (**40**) 含量(mg/g)
2014.0214	S1	0.00	0.33	0.91	0.00
2014.0214	S2	0.89	0.55	0.68	0.00
2014.0214	S3	1.36	9.14	1.51	0.72



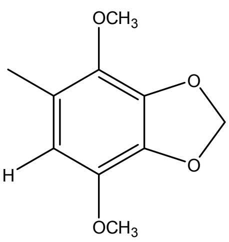
4-AAQB (4-acetyl antroquinonol B) (**76**)



Dehydroeburicoic acid (**25**)



Ergosterol (**37**)



1,4-Dimethoxy-2,3-methylenedioxy-5-methylbenzene(**40**)

批次三:

1. 送件日期 : 2014/04/16

2. 樣品 : K1 牛樟芝　K2 桑黃

3. 檢驗 : 檢驗牛樟芝 8 大指標成分及重金屬含量
   檢驗桑黃重金屬含量

4. 申請單
5. K1 牛樟芝 八大指標成分含量報告書
6. K1 牛樟芝 重金屬檢測報告書
7. K2 桑黃重金屬檢測報告書

批次四:

1. 送件日期 ： 2014/04/17

2. 樣品 ： B1 牛樟芝滴丸

3. 檢驗 ： 檢驗牛樟芝滴丸之 8 大指標成分及重金屬含量
   檢驗牛樟芝滴丸重金屬含量

4. 申請單
5. B1 牛樟芝滴丸 八大指標成分含量報告書
6. B1 牛樟芝滴丸 重金屬檢測報告書

五、結論

根據本實驗測試，大益生技所培育出的牛樟芝，經過精密儀器檢驗得到的報告可知，其牛樟芝含有以下之成分，根據過去文獻對該化合物進行生理活性測試，發現該成分具抗發炎(anti-inflammatory)、抗蟲(anti-insecticidal)和癌細胞毒殺活性(cytotoxic)。未來將有機會發展護肝功能、免疫調節與抗疲勞等功能性健康食品。

所含化合物	文獻上之生理活性
Antcin A	In vitro anti-inflammatory, anti-insecticidal and cytotoxic
Antcin B	In vitro anti-inflammatory, anti-insecticidal and cytotoxic
Antcin C	In vitro anti-inflammatory and cytotoxic
Antcin K	In vitro anti-inflammatory
Antcin H	In vitro anti-inflammatory, anti-insecticidal and cytotoxic
1,4-di-methoxy-2,3-methylenedioxy-5-methylbenzene	In vitro cytotoxic
dehydrosulfurenic acid	In vitro anti-insecticidal and cytotoxic
dehydroeburicoic acid	In vitro anti-inflammatory, anti-insecticidal
4-AAQB (4-acetyl antroquinonol B)	In vitro anti-inflammatory
Ergosterol	

六、參考文獻: