行政院國家科學委員會補助專題研究計畫成果報告

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※　　台灣產欖仁果實及訶黎勒之多酚性成份之研究　　※
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Polyphenolics from the Fruit of Formosan *Terminalia catappa* and *Terminalia chebula*

計畫類別：■個別型計畫　　□整合型計畫

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□赴國外出差或研習心得報告一份

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□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

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中文摘要

為系統性解明台灣產植物之鞣質及其分佈，於本計畫，我們嘗試去探討台灣產欖仁果實及訶黎勒中之多酚性化合物，其結果從欖仁果實，得到六個 gallotannins: 5-*O*-galloyl-(-)-shikimic acid, 6-*O*-galloyl-D-glucose, 1,6-di-*O*-galloyl-β-D-glucose, 3,6-di-*O*-galloyl-D-glucose, 2,3,4,6-tetra-*O*-galloyl-D-glucose 及 1,2,3,4,6-penta-*O*-galloyl-D-glucose; 六個 ellagitannins: 2,3(*S*)-HHDP-D-glucose, corilagin, chebulagic acid, punicalagin, eugeniin 及 acetonylgeraniin; 三個其他型 hydrolysable tannins: chebulinic acid, punicalagin 及 1,3-di-*O*-galloyl-2,4-chebuloyl-β-D-glucose; 二個 phenol-carboxylic acid: gallic acid 及 ellagic acid. 此外，從訶黎勒得到兩個新的 hydrolysable tannins, 3,4,6-tri-*O*-galloyl-D-glucose (**1**) 及 chebulanin (**2**) 以及三個 gallotannins: 1,6-di-*O*-galloyl-β-D-glucose, 1,2,6-tri-/-galloyl-D-glucose, 及 1,2,3,4,6-penta-/-galloyl-D-glucose; 五個 ellagitannins: neochebulagic acid, 1-desgalloyleugeniin, eugeniin, casuarinin 及 1(α)-*O*-galloyl punicalagin.. 另外從訶黎勒葉子，得到四個 ellagitannins: neochebulagic acid, repandusinic acid A, 1-desgalloyleugeniin 及 tercatain; 一個 hydrolysable tannin: neochebulanin.

Abstract

For systemic understanding the tannin and its distribution in Formosan plants, in this project, we try to investigate the polyphenolics from the fruit of Formosan *T. catappa* and *T. chebula*. As the results, from the fruits of *Terminalia catappa*, six gallotannins: 5-*O*-galloyl-(-)-shikimic acid, 6-*O*-galloyl-D-glucose, 1,6-di-*O*-galloyl-β-D-glucose, 3,6-di-*O*-galloyl-D-glucose, 2, 3,4,6-tetra-*O*-galloyl-D-glucose,1,2,3,4,6-penta-*O*-galloyl-D-glucose; six ellagitannins: 2,3(*S*)-HHDP-D-glucose, corilagin, chebulagic acid, punicalagin, eugeniin and acetonylgeraniin; three other hydrolysable tannins: chebulinic acid, punicalagin and 1,3-di-*O*-galloyl-2,4-chebuloyl-β-D-glucose; two phenolcarboxylic acid: gallic acid and ellagic acid were obtained. In addition, two novel hydrolysable tannins, 3,4,6-tri-*O*-galloyl D-glucose (**1**) and chebulanin (**2**) together with three gallotannins: 1,6-di-*O*-galloyl-β-D-glucose, 1,2,6-tri-/-galloyl-D-glucose, and 1,2,3,4,6-penta-/-galloyl-D-glucose; five ellagitannins: neochebulagic acid, 1-desgalloyleugeniin, eugeniin, casuarinin and 1(α)-*O*-galloyl punicalagin were isolated from the fruits of Formosan *Terminalia chebula*.. Four ellagitannins: neochebulagic acid, repandusinic acid A, 1-desgalloyleugeniin and tercatain; one other hydrolysable tannin: neochebulanin were purified from the leaves of *T. chebula.* The structures were elucidated on the basis of chemical and spectroscopic evidence.

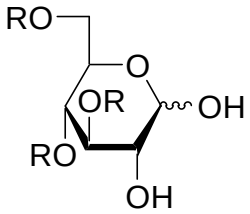
Introduction

Combretaceae, very wide distribution in tropics and subtropical area, is an important source of plant, and used as tan, dye, medicinal plant and garden plant.[1] In the previous course of studies on polyphenolics has lead to find a lot of novel compounds from the genus of *Terminalia*..[2] The studies on the leaf and bark of *T. catappa* have been achieved, and found that the leaf mainly contains ellagitannins and

gallotannins;[3] the bark contains hydrolyzable tannins, condensed tannins and complex tannins, the constitution is very complex;[4] but the fruit is still wait for study. On the other hand, the work of Japanese *T. chebula* was finished, the result show that the fruit contains chebulinic acid and the hydrolyzable tannins come from terchebulic acid unit[5] and the Formosan *T. Chebula* is never being studied. For systemic understanding the tannin and its distribution in Formosan plants, in this project, we try to investigate the polyphenolics from the fruit of Formosan *T. catappa* and *T. chebula*.

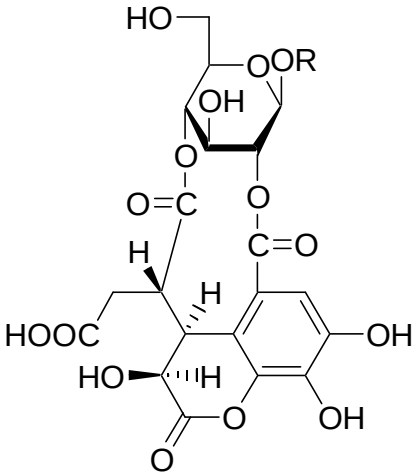
Results and discussion

The 70% aqueous acetone extracts of the fruit of Formosan *T. catappa* and *T. chebula* were respectively subjected to a combination of high porous polystyrene, polydextran and reverse-phase column chromatography with various solvent systems. As the results from *T. catappa*, six Gallotannins: 5-*O*-galloyl-(-)-shikimic acid, 6-*O*-galloyl-D-glucose, 1,6-di-*O*-galloyl-β-D-glucose, 3,6-di-*O*-galloyl-D-glucose, 2, 3,4,6-tetra-*O*-galloyl-D-glucose, 1,2,3,4,6-penta-*O*-galloyl-D-glucose; six Ellagitannins: 2,3(*S*)-HHDP-D-glucose, corilagin, chebulagic acid, punicalagin, eugeniin and acetonylgeraniin; three other Hydrolysable tannins: chebulinic acid, punicalagin and 1,3-di-*O*-galloyl-2,4-chebuloyl-β-D-glucose; two Phenolcarboxylic acid: gallic acid and ellagic acid were obtained. In addition, two novel hydrolysable tannins, 3,4,6-tri-*O*-galloyl D-glucose (**1**) and chebulanin (**2**) together with three Gallotannins: 1,6-di-*O*-galloyl-β-D-glucose, 1,2,6-tri-O-galloyl-D-glucose, and 1,2,3,4,6-penta-O-galloyl-D-glucose; five Ellagitannins: neochebulagic acid, 1-desgalloyleugeniin, eugeniin, casuarinin and 1(α)-*O*-galloyl punicalagin were isolated from the fruits of Formosan *T. chebula*. On the other, Four Ellagitannins: neochebulagic acid, repandusinic acid A, 1-desgalloyleugeniin and tercatain; one other Hydrolysable tannin: neochebulanin were purified from the leaves of *T. chebula.* The structures were elucidated on the basis of chemical and spectroscopic evidence.[4,5]



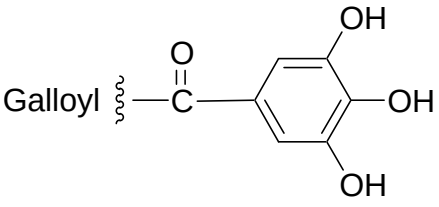
R: Galloyl

1



2: R= Galloyl

2a: R=H



Experimental

^{1}H and ^{13}C NMR spectra were measured with taken with TMS as an internal standard; chemical shifts are give on a δ (ppm) scale. Column chromatography was carried out with Sephadex LH-20 (25-100 μ, Pharmacia Fine Chemical), MCI-gel CHP20P (75-150 μ, Mitsubishi Chemical Industries), Fuji-Gel ODS-G3 (43-65 m, Fuji Gel Hanbi). Cosmosil C18-OPN (75mm, Nacalai). The fresh fruit of plants were collected in Ping-tung, Taiwan (Jul.), and verified by Dr. Feng-Chi Ho (Taiwan Forestry Research Institute, Heng-Chun, Ping-Tung, Taiwan). The fresh fruit (4.2 Kg) was extracted 2 times with 80 % aqueous acetone at room temperature, and the extract was concentrated under reduced pressure, the resulting brown precipitated was subjected to Sephadex LH-20 column chromatography with water containing increasing amounts of MeOH and finally with a mixture of water-acetone (1:1).

3,4,6–Tri-*O*-galloyl-D-glucose (1)

White crystal power, mp 194～196°C. [á] $_{D}^{25}$ 77.4° (c = 1.1, MeOH). Anal. Calcd for $C_{27}H_{24}O_{18}$ • $3H_2O$: C, 46.95; H, 4.34. Found: C, 46.58; H, 4.38. Negative FAB-MS m/z: 635 $(M-H)^-$. ^{1}H-NMR (acetone-d_6+D_2O) ä: 4.20 (dd, J = 4, 12 Hz, H-6), 4.40 (d, J = 12 Hz, H-6), 4.87 (d, J = 8 Hz, â-H-1), 5.75 (t, J = 9 Hz, H-3), 7.04, 7.05, 7.07, 7.16, 7.17 (each s, galloyl-H). ^{13}C-NMR (acetone-d_6 + D_2O) ä : 63.5 (C-6), 68.6, 70.0, 72.0, 72.8, 74.0, 74.6, 75.9 (C-2, 3, 4, 5), 93.5 (á-C-1), 98.3 (â-C-1), 110.1 (galloyl C-2, 6), 120.8, 121.5 (galloyl C-1), 138.8, 139.1 (galloyl C-4), 145.8 (2C, galloyl C-3, 5), 166.3, 166.5(-COO-).

Methylation of **1.** A mixture of **1** (30 mg) , $(CH_3)_2SO_4 \cdot K_2CO_3$ in acetone was refluxed. Purification of the product over silica gel [benzene-acetone (4:1 v/v)] to give **1a** (14.3 mg) & **1b** (6.3 mg). **1a**: $[\alpha]_D^{20}$ +33.6 (c=0.7, $CHCl_3$), Anal. Calcd for $C_{37}H_{44}O_{18} \cdot 1/2H_2O$: C, 57.55; H, 5.64; Found: C, 57.46; H, 5.92. FD-MS m/z 776 $[M]^+$. ^{1}H-NMR ($CDCl_3$) ä: 4.35 (2H, m, H-5, 6), 4.70 (dd, J = 6, 14 Hz, H-6), 4.95 (d, J = 4 Hz, H-1), 5.51 (t, J = 10 Hz, H-4), 5.66 (t, J = 10 Hz, H-3). **1b:** $[\alpha]_D^{20}$ -21.4 ° (C = 0.4, $CHCl_3$). Anal. Calcd for $C_{37}H_{44}O_{18} \cdot 1/2H_2O$: C, 57.55; H, 5.64. Found: C, 57.10; H, 6.02. FD-MS m/z: 776 $[M]^+$. ^{1}H-NMR ($CDCl_3$) ä: 4.35 (d, J = 13 Hz, H-6), 4.67 (dd, J = 4, 13 Hz, H-6), 4.50 (d, J = 8 Hz, H-1), 5.50 (t, J = 7 Hz, H-4), 5.55 (t, J = 7 Hz, H-3). Acid Hydrolysis of **1**.. A solution of **1** (11.1 mg) in 1N H_2SO_4 (1 ml) was heated at 95 ℃ for 4 h. After cooling, the reaction mixture was analyzed by TLC on cellulose [*n*-BuOH-pyridine-H_2O (6:4:3)], which showed a sport (*Rf.* 0.4) corresponding to glucose. Further purification with column to yield gallic acid.

Chebulanin (2)

Off-whit amorphous powder (H_2O), $[\alpha]_D^{17}$ +52.8° (C = 1.1, MeOH). Anal. Calcd for $C_{27}H_{24}O_{19} \cdot 5/2H_2O$: C, 46.49; H, 4.19. Found: C, 46.12; H, 4.06. Negative FAB-MS m/z: 651 $[M-H]^-$: 551, 459, 367. ^{1}H-NMR (acetone-d_6 + D_2O) ä: 2.20 (2H, d, J = 8 Hz, Che-H-5'), 3.90 (1H, br t, J = 7 Hz, Che-H-4'), 4.00-4.44 (m, H-3, 4, 6), 4.86 (1H, br s, H-4), 4.89 (1H, d, J = 7 Hz, Che-H-2'), 5.18 (1H, dd, J=2, 7 Hz, Che-H-3'), 5.26 (1H, br, s, H-2), 6.40 (1H, d, J = 3 Hz, H-1), 7.20 (2H, s, galloyl-H), 7.50 (1H,

s, Che-3-H). ^{13}C-NMR (acetone-d_6 + D_2O) ä: 30.3 (C-5'), 39.4 (C-4'), 41.0 (C-3'), 61.2, 62.8, 66.6, 71.5, 73.5 (glc. C-2, 3, 4, 5, 6), 79.2 (C-2'), 92.4 (glc. C-1), 110.3 (galloyl C-2, 6), 115.8, 116.9, 119.0 (C-1'', 2'', 3''), 120.2 (galloyl C-1), 139.5, 139.7, 140.9 (C-4'', 5'', galloyl C-4), 146.0 (galloyl C-3, 5), 146.5 (C-6''), 165.6, 166.0, 169.8, 173.5, 174.0 (-COO-). Tannase Hydrolysis of **2** A solution of **2**（50 mg）in H_2O was shaken with tannase at room temperature for 30 min. Then the reaction mixture was purified by Sephadex LH-20 (H_2O : MeOH) (1:0 - 0:1), to give gallic acid (14.3 mg) and **2a** (31 mg). **2a**: $[\alpha]_D^{22}$ +28.3° (c = 1.0, H_2O-acetone, 1:1). Anal .Calcd for $C_{20}H_{20}O_{15} \cdot 9/2H_2O$: C, 41.31; H, 3.46. Found: C, 41.33; H, 3.98. Negative FAB-MS m/z: 499 $[M-H]^-$, 367, 265. ^{1}H-NMR (acetone-d_6 + D_2O) ä: 2.23 (2H, d, J = 8 Hz, Che-H-5'), 3.45-3.86 (3H, m, H-3,6), 4.82 (1H, d, H-4), 4.92 (1H, d, J = 8 Hz, H-5), 5.25 (1H, m, H-2), 5.44 (1H, d, J = 2 Hz, H-5), 7.58 (1H, s, Che-H-3).

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