

Plant Origin and Anti-bacterial Activity of Taiwanese Green Propolis



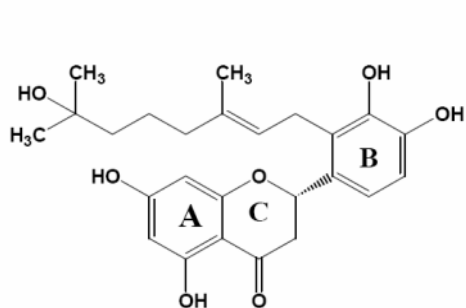
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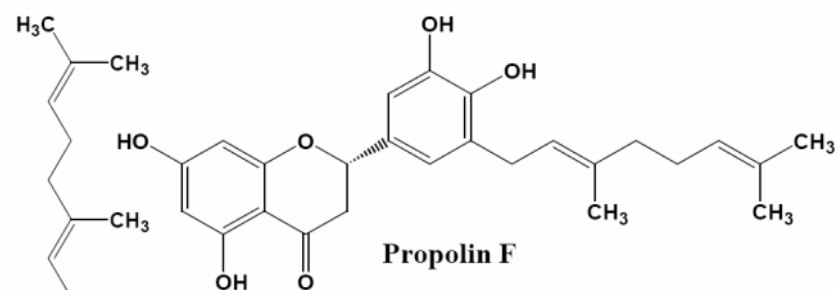
² Dept. of Leisure Management, Minghsin University of Science and Technology, Taiwan

Taiwanese Propolis

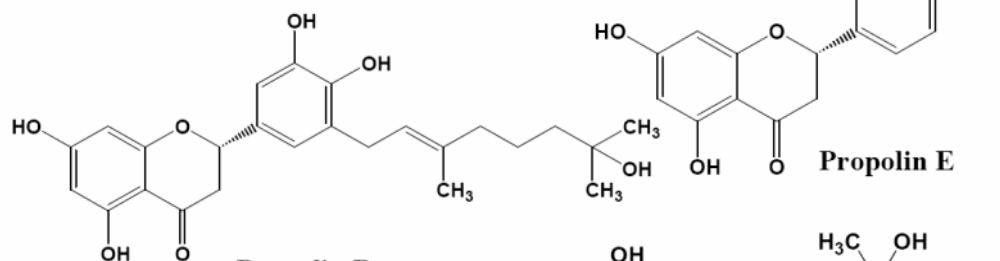
- Specific – **Pacific type** (Bankova, 2005)
- Active compound of Taiwanese propolis (Chen et al., 2003)
 - Prenylflavanones – named “propolin”
 - Propolin A – J
 - Propolin C, D, F and G are the major component
- Biological activities:
 - anti-tumor; DPPH scavenging; anti-microbial; anti-mutagenicity; HDAC (histone deacetylase) inhibitor (Chen et al. 2003, 2004a, 2004b, 2006, 2007, 2008a, 2008b; Lu et al. 2003, 2005; Yang et al. 2006; Huang et al. 2007; Weng et al. 2007)



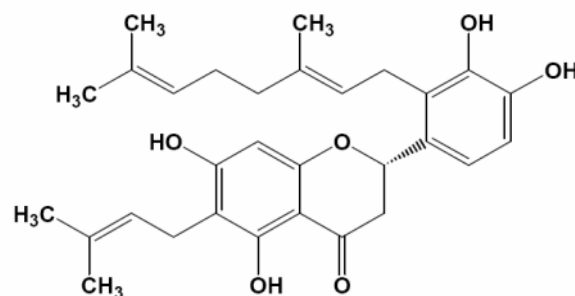
Propolin A



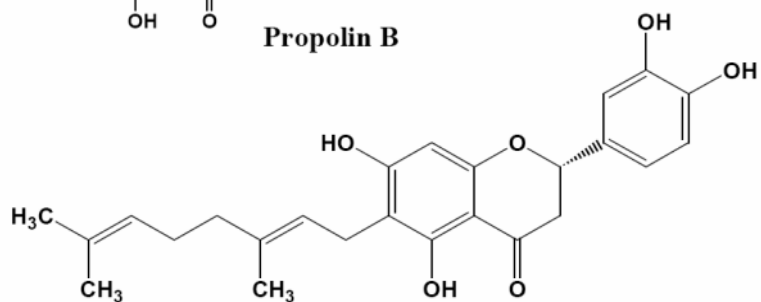
Propolin F



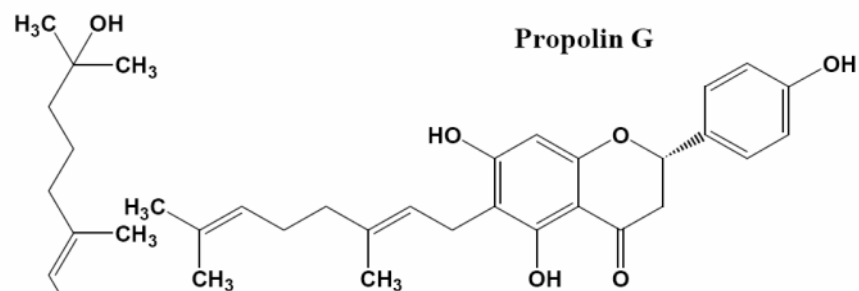
Propolin B



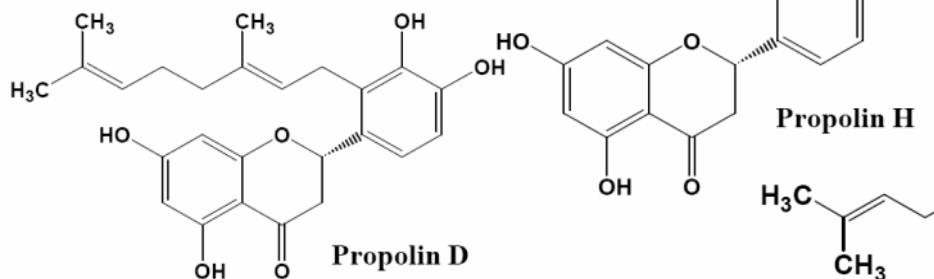
Propolin G



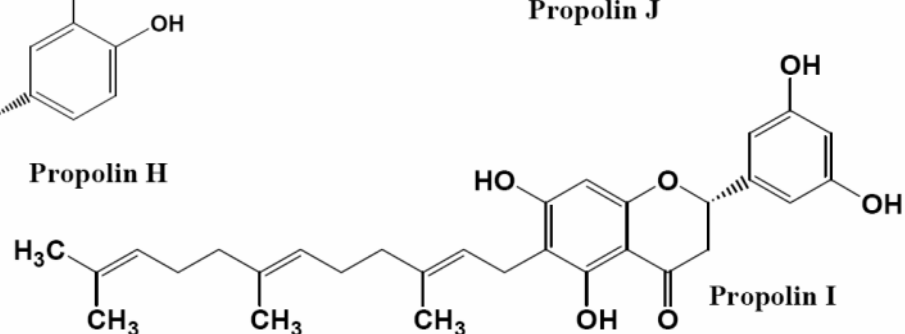
Propolin C



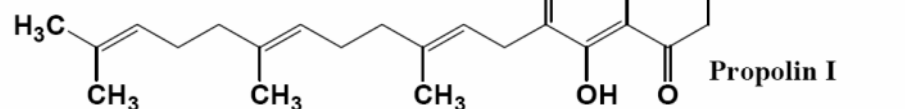
Propolin J



Propolin D



Propolin H



Propolin I

Classification of Taiwanese propolis

- Seasonally effect of Taiwanese green propolis:

- TW-I : May to July
- TW-II : August to September
- TW-III : October to November

(Chen et al., 2008)



Green Brown- green Dark brown

- Taiwanese Green propolis (TW-I) is the best
 - higher yield ($71.5 \pm 6.0\%$), higher total phenolic , propolins
 - best bio-activities

What's the plant origin of TW-I propolis?

- More than 30 plants had been evaluated
- Only the *Macaranga tanarius* contain propolins
- Extracts of new leaves and buds contain a high amount of propolin C, D and G

(Chen, 2007 in 40th Apimondia)

- Ratio of propolins between TW-I propolis and leaves of *M. tanarius* are not similar

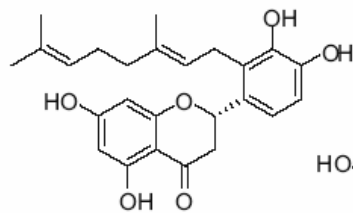


Propolis in Okinawa, Japan

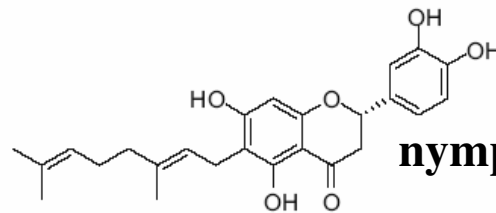
(Kumazawa et al., 2008)

- Major component is similar as Taiwanese Green propolis
- studied the collecting behavior of honeybee in Okinawa and pointed out the plant source is *M. tanarius* (the surface matrix of the fruit)
 - Prenylflavanones of propolis from Okinawa, Japan:

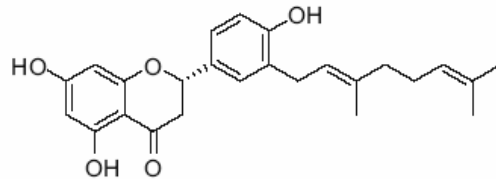
**nymphaeol-B
(propolin D)**



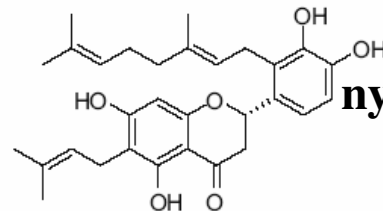
nymphaeol-A (propolin C)



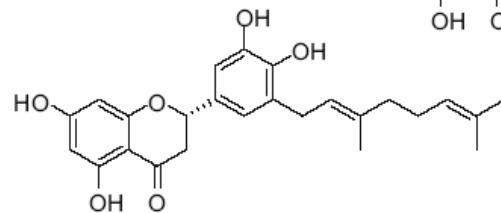
**3'-geranyl-naringenin
(propolin H)**



nymphaeol-C (propolin G)



isonymphaeol-B (propolin F)



Fruit of *M. tanarius*

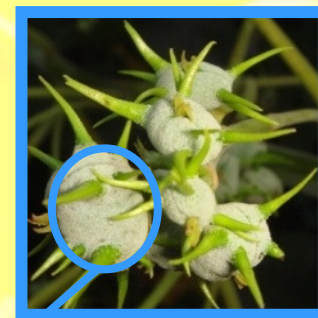


Macaranga tanarius

- *M. tanarius* is well known as a pioneer tree.
 - This plant can be found throughout eastern and southern Asia.
 - Fruiting phase and harvest of TW-I propolis :May to July.
 - Bioactivities of leaves of *M. tanarius*
 - Antioxidant (Matsunami et al., 2006 ; Phommart et al., 2005)
 - Antimicrobial (Lim et al., 2008)
- ➡ *1. Surface material of M. tanarius fruit is origin of TW-I propolis?*
- 2. Other regions of the plant still contain propolins and its activity?*



Flower



Material on the fruit : hand-scratched

Leaf : 4~5 leaves beneath the fruit



Outer fruit : extracted with MeOH



Stalk



Inner fruit : the whole fruit were extracted with MeOH after homogenizing

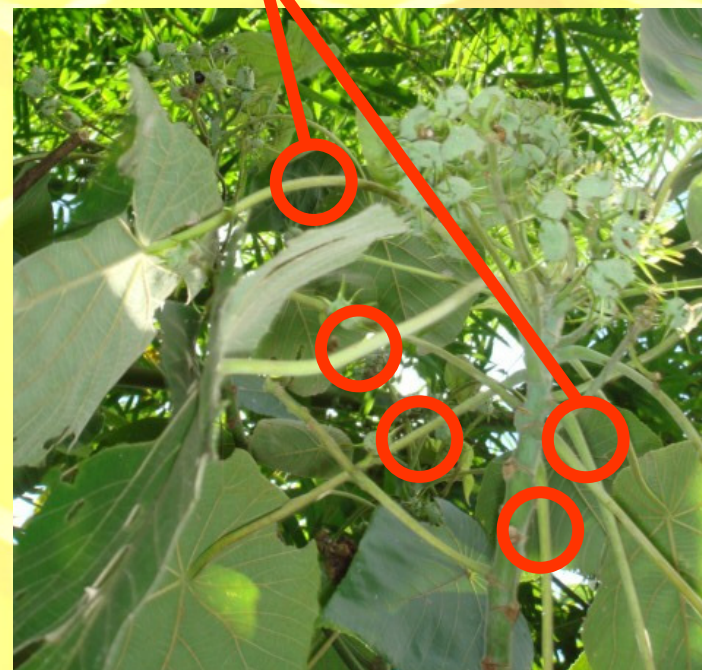


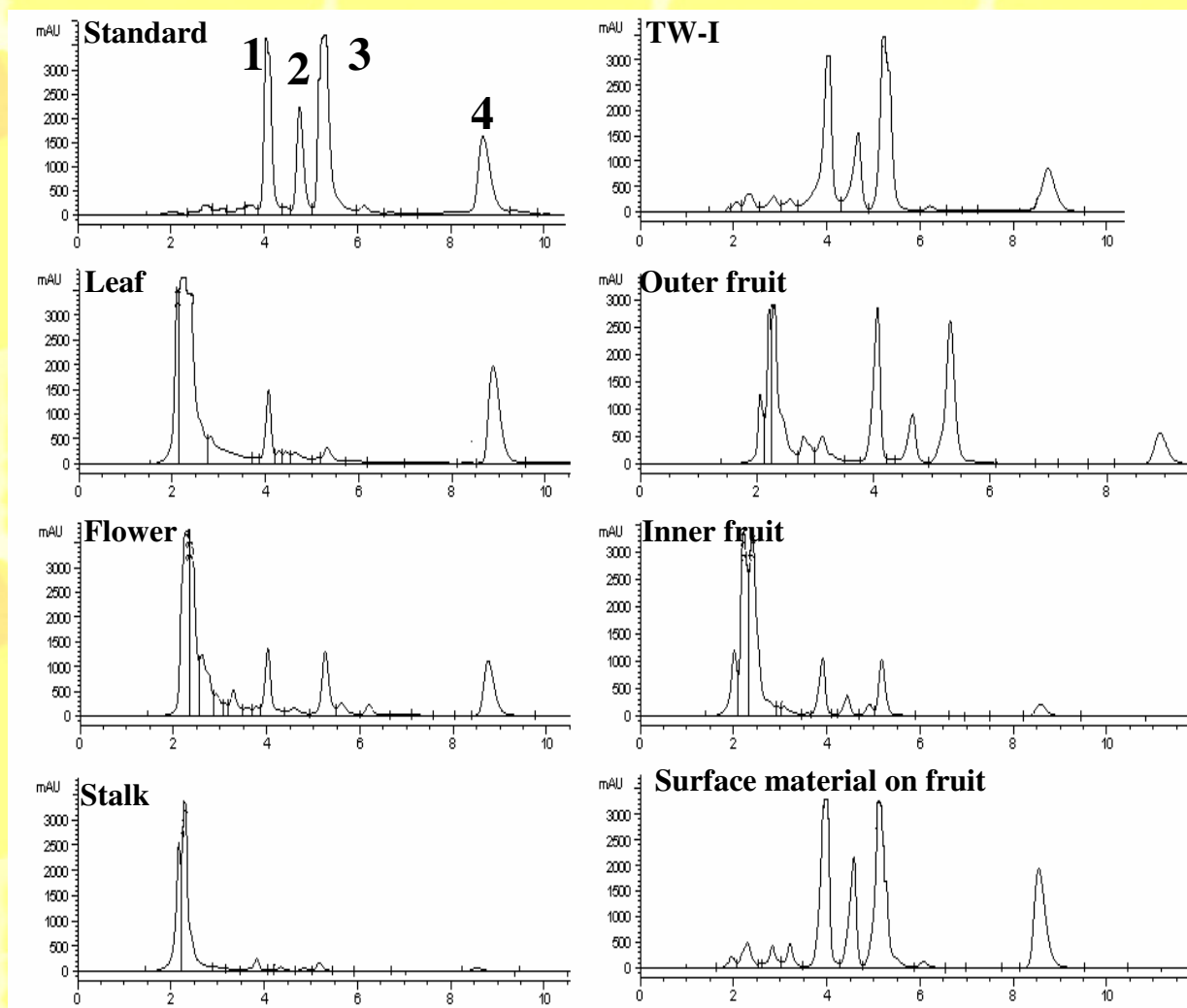
Table 1. Dry matter yield (%) and propolin content (mg/g) in different location of *Macaranga tanarius* extracted by methanol¹

Location of extracts	Yield	Propolin C	Propolin D	Propolin F	Propolin G	Propolin C+D+F+G
Leaf	19.75±0.96c	1.60±0.09f	3.65±0.07d	1.52±0.04d	16.38±0.58c	23.14±0.74c
Flower	10.00±0.82d	2.31±0.12e	1.97±0.09f	0.83±0.04f	4.10±0.18d	9.21±0.42d
Stalk	6.75±0.50f	0.26±0.01g	0.32±0.05g	0.47±0.10g	0.44±0.10f	1.49±0.25e
Outer fruit	8.25±0.50e ²	8.96±0.30c	6.37±0.27c	2.95±0.07c	4.04±0.20d	22.31±0.83c
Inner fruit	8.00±0.00e	3.33±0.09d	2.87±0.06e	1.33±0.03e	1.77±0.06e	9.30±0.24d
Surface material on fruit	62.25±0.50a	178.36±1.31a	122.41±0.68a	69.50±1.16a	151.43±1.99a	521.69±3.95a
TW-I extract ³	59.75±1.26b	133.07±8.78b	69.27±3.98b	39.29±2.68b	91.82±6.63b	333.45±21.52b

¹10g substance were extracted by 100mL methanol, extracts were finally made up to 100mL.

²Values are mean ± s.d. Group means in the same column followed by different letters are significantly different by LSD test (P < 0.05).

³Taiwanese green propolis was extracted with methanol.



1: Propolin D

2: Propolin F

3: Propolin C

4: Propolin G

Figure 1. HPLC profiles of propolins. The separation conditions were as follows: column, ZORBAX SB-C18 (4.6 × 250 mm; made in USA); mobile phase, methanol/water 88.8:11.2 v/v; flow rate, 1mL/min; detection, UV at 280 nm; injection volume, 20μL.

Table 2. Antibacterial activity of extracts from the surface material on fruits of *M. tanarius*

Bacteria strains	<i>M. tanarius</i>		TW-I propolis	
	MIC	MBC	MIC	MBC
	(µg/mL)		(µg/mL)	
<i>S. aureus</i> (BCRC 10780)	5	10	20	20
<i>S. aureus</i> (BCRC 10781)	5	10	20	20
<i>S. aureus</i> (BCRC 10451)	10	10	20	40
<i>B. subtilis</i>	1.25	2.5	10	20
<i>B. cereus</i>	1.25	2.5	20	20
<i>E. coli</i>	> 640	> 640	> 640	> 640
<i>P. aeruginosa</i>	> 640	> 640	> 640	> 640

Table 3. MIC and MBC ($\mu\text{g/mL}$) of extracts of different location of *M. tanarius* against *S. aureus* and *E. coli*

Extracts	<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>
	MIC	MBC	MIC
Leaf	40	80	>640
Flower	40	80	>640
Stalk	>640	>640	>640
Outer fruit	20	40	>640
Inner fruit	40	80	>640
Surface material on fruit	5	10	>640
TW-I propolis	20	40	>640

Table 4. Half-inhibition (IC⁵⁰) of extracts of different location of *M. tanarius* in scavenging DPPH radicals

Extracts	IC₅₀ (µg/ml)
Leaf	9.67±0.55^{b2}
Flower	14.30±0.84^c
Stalk	21.12±3.09^d
Outer fruit	20.32±4.12^d
Inner fruit	15.76±1.95^c
Surface material on fruit	7.49±0.62^a
TW-I propolis	21.58 ± 0.25^d

¹ IC₅₀, the efficient concentration decreasing initial DPPH concentration by 50%, was obtained by the interpolation from linear regression analysis.

²Values are mean ± s.d. Group means in the same column followed by different letters are significantly different by LSD test (P < 0.05).

Discussion and Conclusion

- Plant source of Taiwanese green propolis
 - The surface material of the fruit of *M. tanarius*, same as Okinawa, Japan.
 - The best dry matter yield: $62.25 \pm 0.50\%$, extract of material on fruit.
 - Performance of extract of stalk was the worst.

Bioactivity

- **Anti-bacterial activity**
 - Surface material from fruit of *M. tanarius* exhibited powerful antibacterial activity.
 - Effect of stalk extract on the strains: slightly.
 - *S. aureus* 10781 was the most sensitive.
 - All extracts had no activity against *E. coli*.

Bioactivity

- **DPPH scavenging activity**

- Surface material from fruit of *M. tanarius* existed fine DPPH scavenging activity, 7.49 ± 0.62 ($\mu\text{g/ml}$).
- Extract of leaf : chlorophylls (Ursula et al., 2005)
- Taiwanese honey bees are Smart!!

They can find out the special plant (*M. tanarius*) and its exact location (surface material of fruit) to make up the TW-1 propolis