

# **Basic research of Brazilian propolis: major constituents and *in vitro* anti-tumor activities**

**Taichi Mitsui\*, Shigemi Tazawa, Sho Hotta,  
Kenji Kato, and Kenji Ichihara**

**Nagaragawa Research Center, API Co., Ltd.,  
Nagara 692-3, Gifu, Japan**



**<http://www.api3838.co.jp/en/index.html>**

# Agenda

## 1. Introduction

## 2. Propolis from the state of Parana

- Constituents
- *in vitro* anti-tumor activity

## 3. Propolis from the state of Bahia

- Constituents
- *in vitro* anti-tumor activity

## 4. Summary and Conclusion

# Areas of Brazilian Propolis



**Red Propolis**  
**(Alagoas, Bahia)**



**Bahia propolis**  
**(Bahia)**



**Green propolis**  
**(Minas Gerais)**



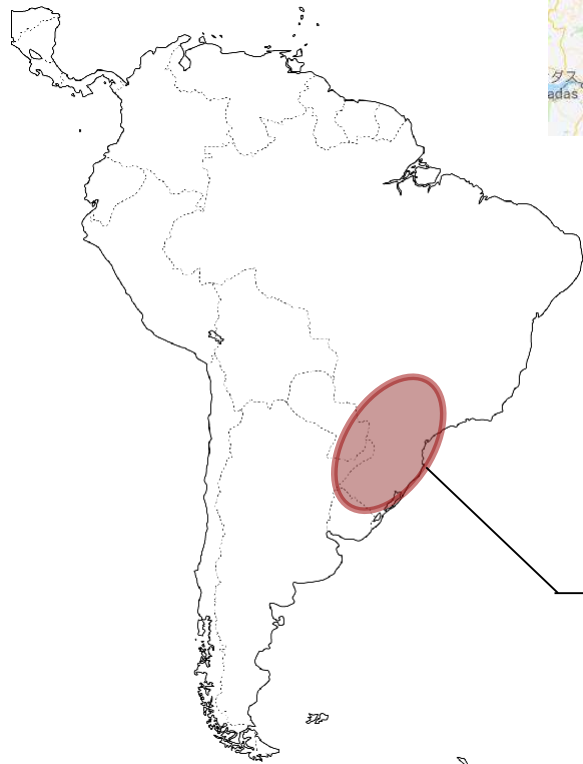
**Brown propolis**  
**(Parana)**



# Propolis from the state of Parana

<https://www.google.co.jp/maps>

<http://www.roconsulboston.com>



Parana state in Brazil  
199,314 km<sup>2</sup>

Romania  
238,397 km<sup>2</sup>

**Brown Propolis**  
**Parana**



# Analysis of Parana Propolis



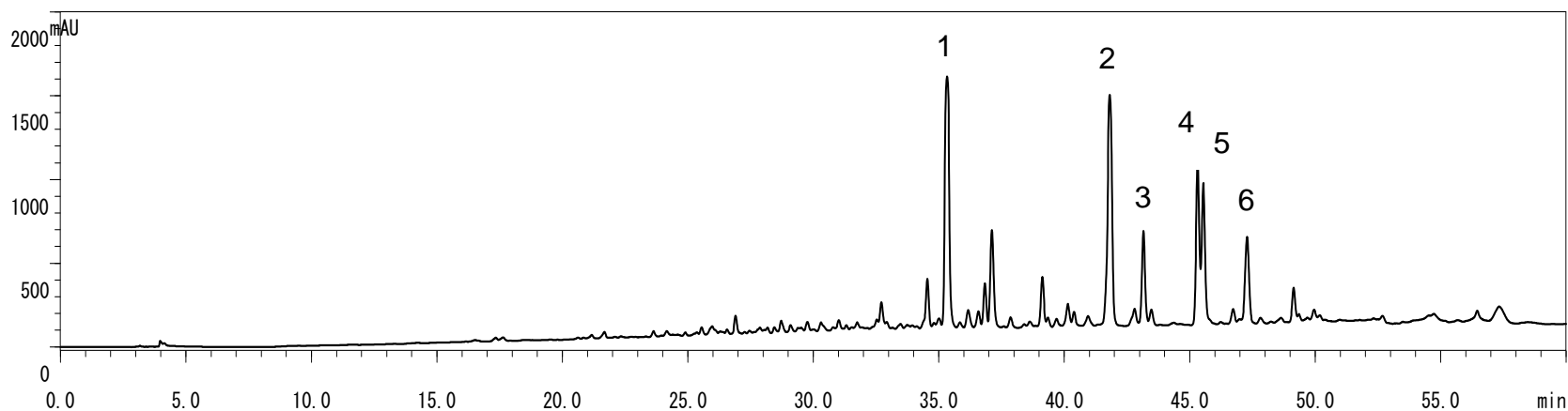
**Extracts with 95% ethanol**

**《HPLC》**

**Solvent: Gradient mixture of Acetonitrile-H<sub>2</sub>O  
(0.1% Phosphoric acid)**

**Flow: 1.0 mL/min, Detect: 205 nm**

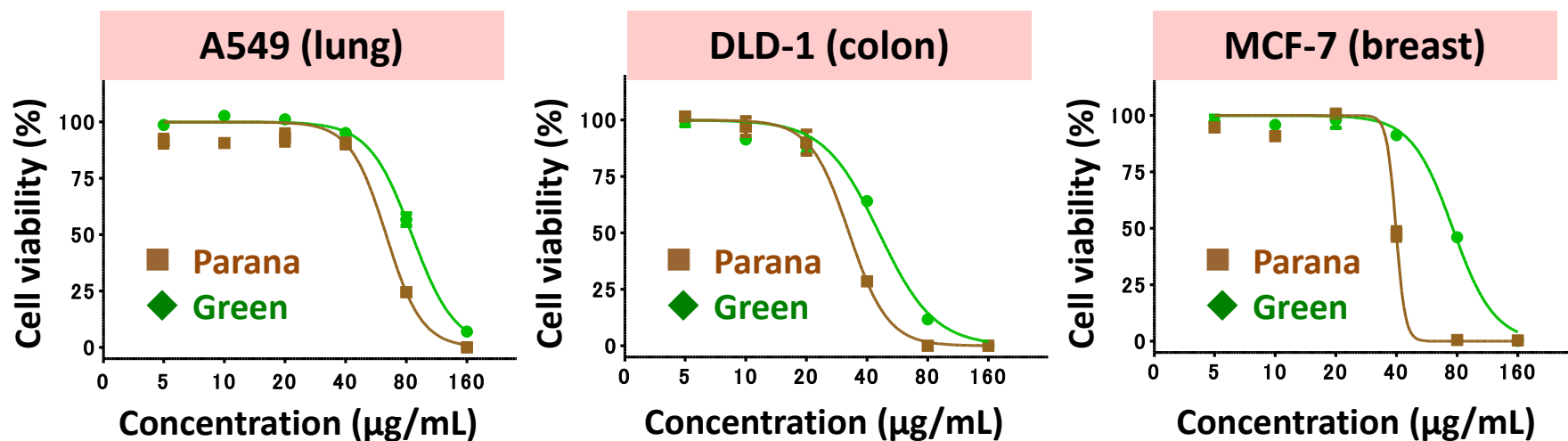
**Column: Shim-pack CLC-ODS 6 mm x 150 mm (Shimadzu Corp)**



*S. Tazawa et al., Natural Product Communications, 11(2), 201-205 (2016)*

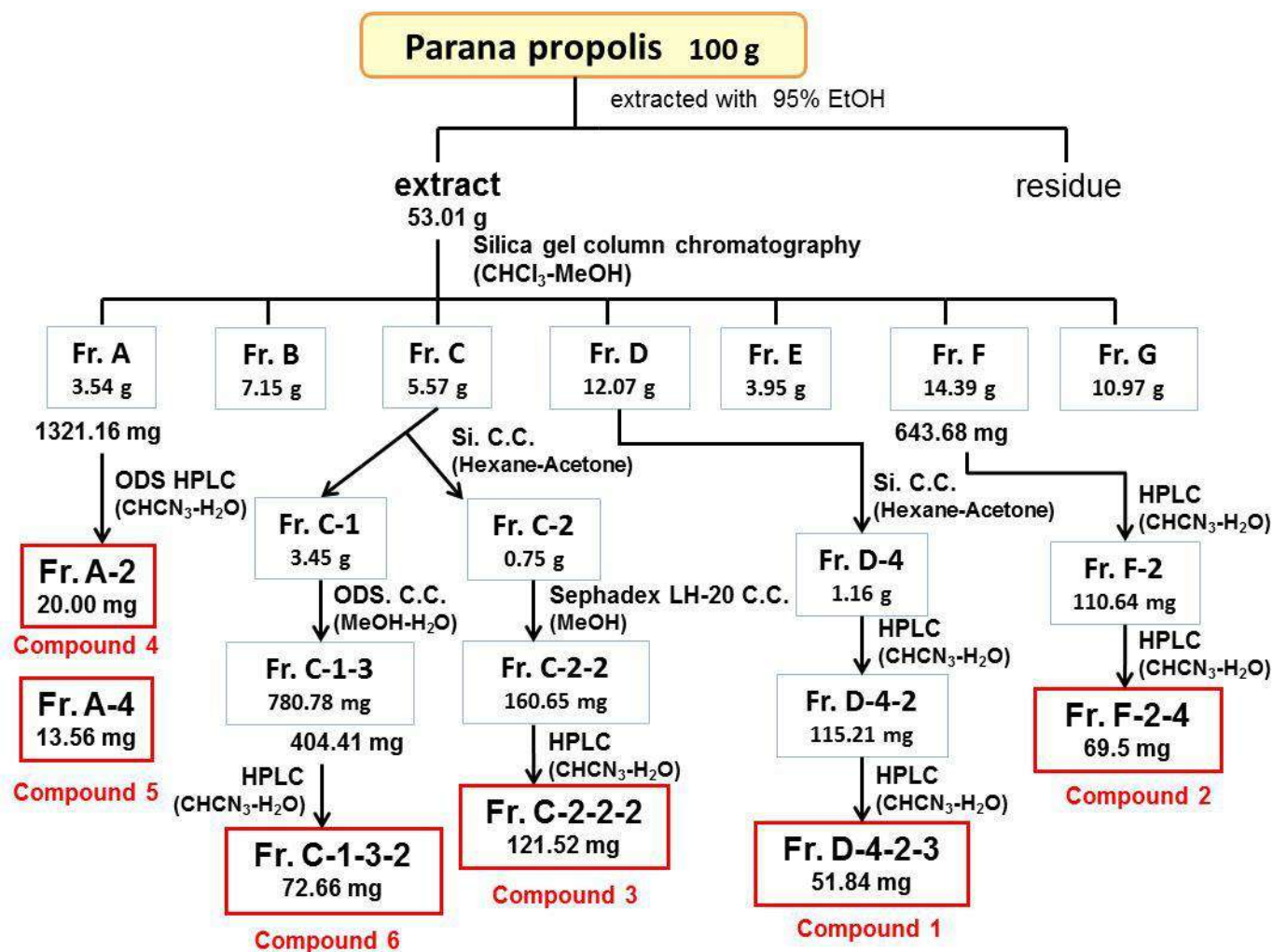
# Anti-tumor activities of the ethanolic extracts

The tumor cells were cultured and then were treated with the extracts for 24 h. Growth inhibition was evaluated by quantifying living cells using the Cell Counting Kit-8 , and IC<sub>50</sub> values were determined in all tumor cell lines tested.



IC <sub>50</sub> (µg/mL)	A549 (lung)	DLD-1 (colon)	MCF-7 (breast)
Parana Propolis	62.6	33.3	39.3
Green Propolis	85.6	42.8	77.6

**Chromatographically separation**





# Determination of the structures of the isolated compounds

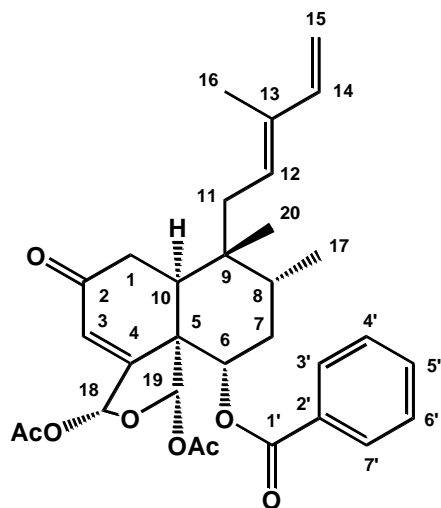
**IR, UV**  
Functional group



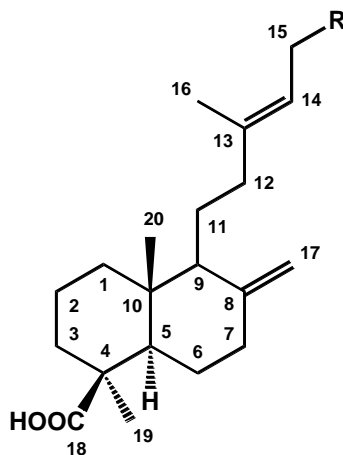
**(HR) ESI-MS**  
Molecular Weight



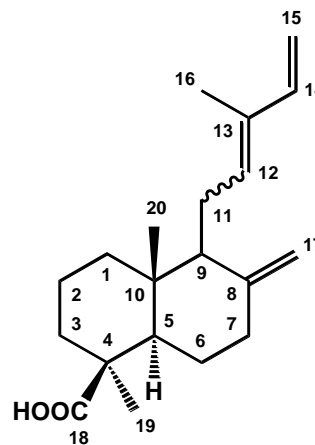
**1D & 2D-NMR**  
Chemical Structure



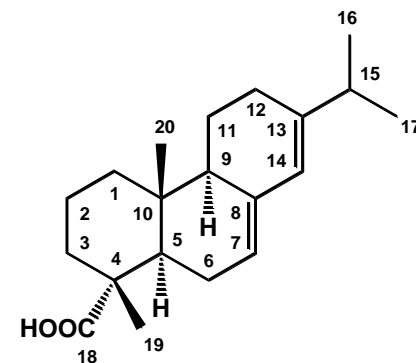
**Novel compound 1**



**Compound 2 : R = OH**  
**Compound 3 : R = OAc**



**Compound 4 :  $\Delta^{12, 13} = E$**   
**Compound 5 :  $\Delta^{12, 13} = Z$**



**Compound 6**



# Anti-tumor activities of the isolated compounds

Compounds	IC <sub>50</sub> (μM)			
	LNCaP (prostate)	MCF-7 (breast)	DLD-1 (colon)	A549 (lung)
<b>Novel clerodane diterpene (1)</b>	<b>6.2</b>	<b>6.3</b>	<b>6.8</b>	<b>12.7</b>
Isocupressic acid (2)	109.6	176.6	139.4	>200.0
15-Acetoxyisocupressic acid (3)	101.1	133.1	139.1	153.6
( <i>E</i> )-Communic acid (4)	110.7	164.6	121.2	169.5
( <i>Z</i> )-Communic acid (5)	130.6	173.9	139.5	>200.0
Abietic acid (6)	97.1	174.8	150.3	178.7

S. Tazawa *et al.*, *Natural Product Communications*, 11(2), 201-205 (2016)

- A novel clerodane-type diterpene (1) showed a marked inhibition of cell growth, and its activity was approximately 20 times more potent than those of other diterpenes.

# Propolis from the state of Bahia



[https://en.wikipedia.org/wiki/Salvador,\\_Bahia](https://en.wikipedia.org/wiki/Salvador,_Bahia)

# Article of Newspaper (in Brazil, 1998)

"Propolis prevents the human body from invasion of tumors"

## SAÚDE Pesquisas mostram que a propolis pode atuar na prevenção e no tratamento de vários tipos de tumores Propolis pode proteger corpo de tumores

### Como foi feito o estudo

Para saber qual o efeito da propolis de diferentes regiões do país, foram coletados 400 amostras de propolis de 12 tipos diferentes de regiões do país. As amostras foram analisadas em laboratório de química, onde foram identificados os compostos químicos e a concentração de cada um deles. Os resultados foram publicados na revista "Ciência & Saúde" da Universidade Federal de Pernambuco.

**Porcentagem de inibição de tumores malignos\***

Local da origem	Número da amostra**	Câncer de mama	Câncer de intestino	Câncer de fígado	Câncer de estômago	Câncer de pulmão
Rio Grande do Sul	5	22	30	-	30	30
Ceará	3	37	22	12	31	31
Pernambuco	5	43	31	12	43	43
Bahia	8	97	95	94	96	96
Bahia	11	85	77	74	80	80
Bahia	12	88	79	72	77	77
Bagé	1	50	48	9	37	37
Bagé	3	14	39	8	18	18
Peiotas	1	53	45	12	31	31
Pelotas	3	50	47	13	33	33

\*O cálculo da porcentagem foi feito tomando-se como base a porcentagem de inibição da droga Etoposide, a mais forte contra câncer existente no mercado. Esse é um método padrão de análise antitumoral, desenvolvido pelo Instituto Nacional do Câncer dos EUA.

da composição química). Do total, foram

provenientes da Bahia, do Rio Grande do

tamanho dos tumores

**Porcentagem de inibição de tumores malignos\***

Local da origem	Número da amostra**	Câncer nasofaríngeo	Câncer de intestino	Câncer renal	Câncer de mama
Rio Grande do Sul	5	22	30	-	30
Ceará	3	37	22	12	31
Pernambuco	5	43	31	12	43
Bahia	8	97	95	94	96
Bahia	11	85	77	74	80
Bahia	12	88	79	72	77
Bagé	1	50	48	9	37
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Peiotas	1	53	45	12	31
Pelotas	3	50	47	13	33

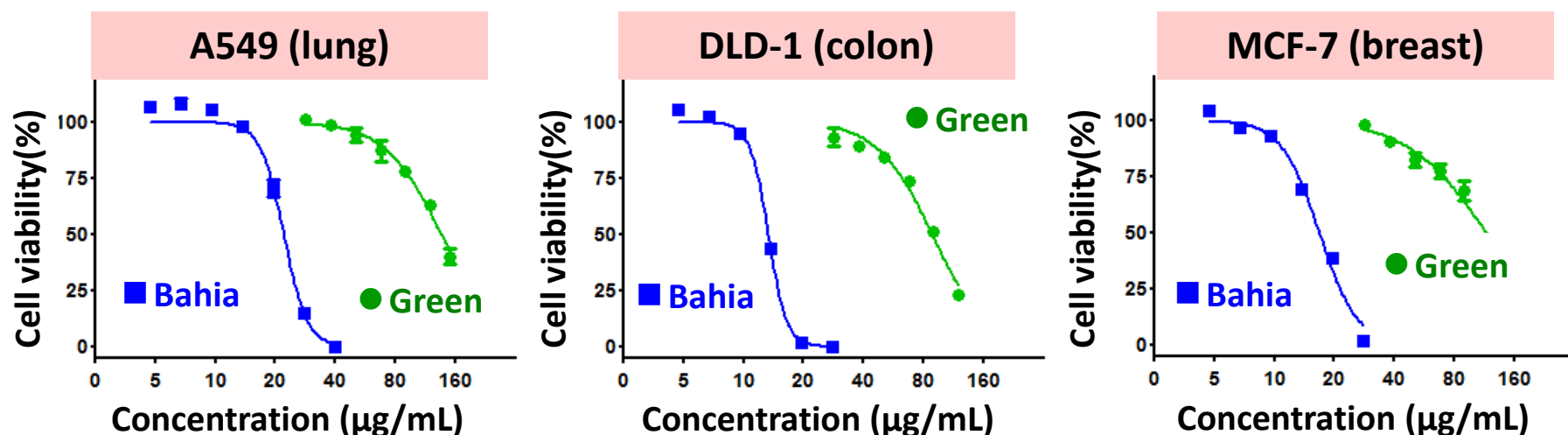
\*O cálculo da porcentagem foi feito tomando-se como base a porcentagem de inibição da droga Etoposide, a mais forte contra câncer existente no mercado. Esse é um método padrão de análise antitumoral, desenvolvido pelo Instituto Nacional do Câncer dos EUA.

Dr. Park Yong Kun ...

Four hundred Brazilian propolis have been grouped into 12 chemical types according to their geographical origin. As the results, **Bahia propolis showed the most potent inhibitory activities against several cancer cell lines.**

# Anti-tumor activities of the ethanolic extracts

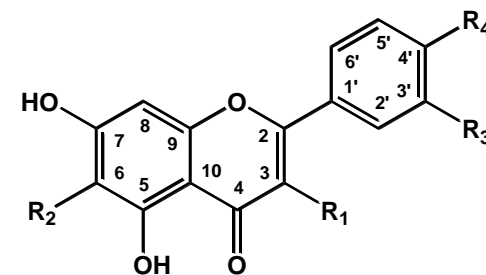
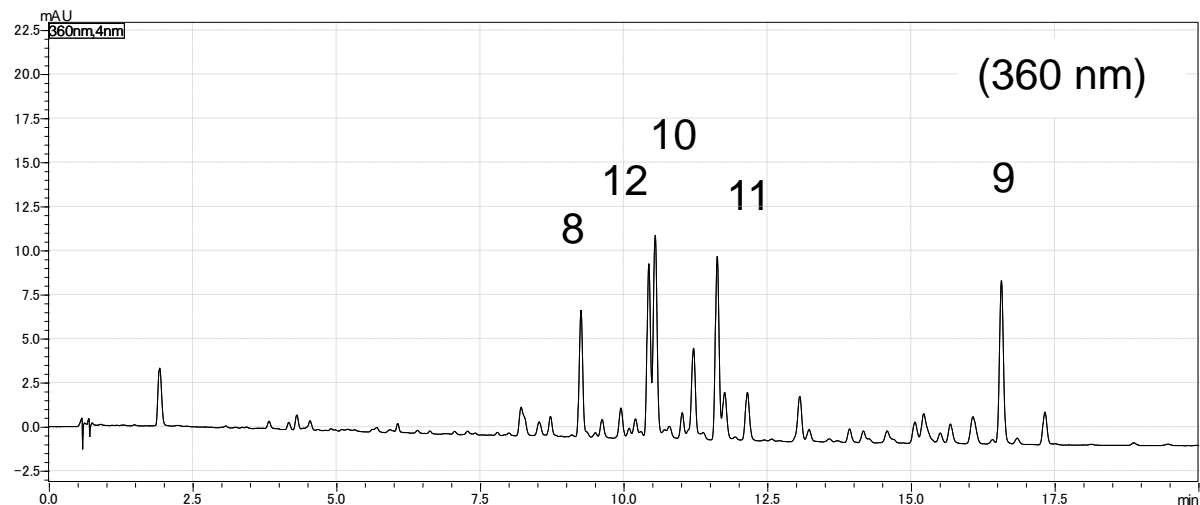
The tumor cells were cultured and then were treated with the extracts for 24 h. Growth inhibition was evaluated by quantifying living cells using the Cell Counting Kit-8, and IC<sub>50</sub> values were determined in all tumor cell lines tested.



IC <sub>50</sub> (µg/mL)	A549 (lung)	DLD-1 (colon)	MCF-7 (breast)
Bahia Propolis	21.7	13.8	16.8
Green Propolis	138.1	92.0	113.7

T. Mitsui *et al.*, *Biosci. Biotech. and Biochem.*, 82(3), 417-421 (2018)

# Analysis of Bahia Propolis



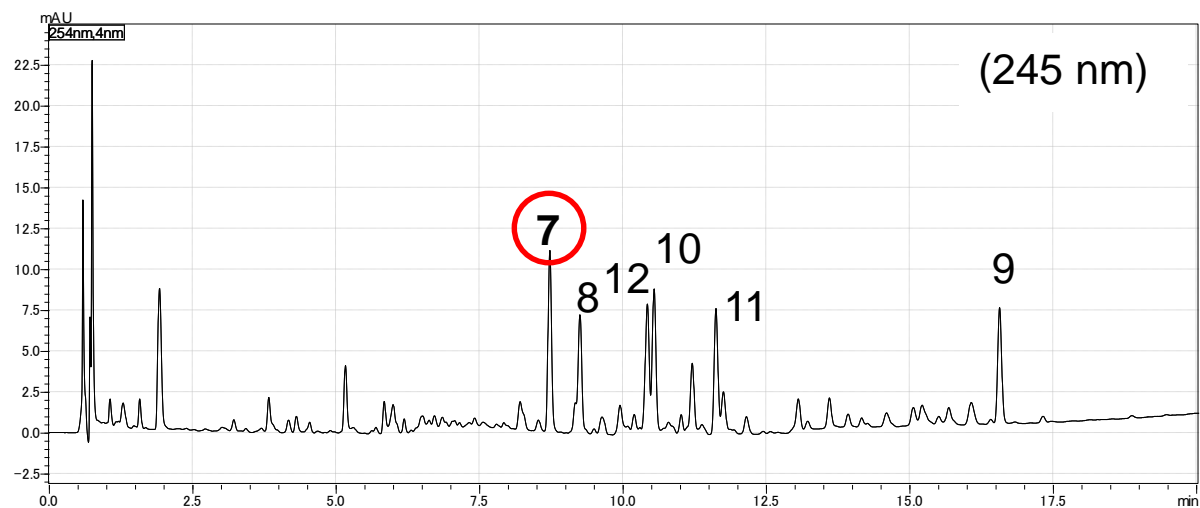
**8:** R<sup>1</sup>=OCH<sub>3</sub>, R<sup>2</sup>=H, R<sup>3</sup>=R<sup>4</sup>=OH

**9:** R<sup>1</sup>=R<sup>2</sup>=R<sup>4</sup>=OCH<sub>3</sub>, R<sup>3</sup>=H

**10:** R<sup>1</sup>=R<sup>2</sup>=OCH<sub>3</sub>, R<sup>3</sup>=H, R<sup>4</sup>=OH

**11:** R<sup>1</sup>=R<sup>4</sup>=OH, R<sup>2</sup>=OCH<sub>3</sub>, R<sup>3</sup>=H

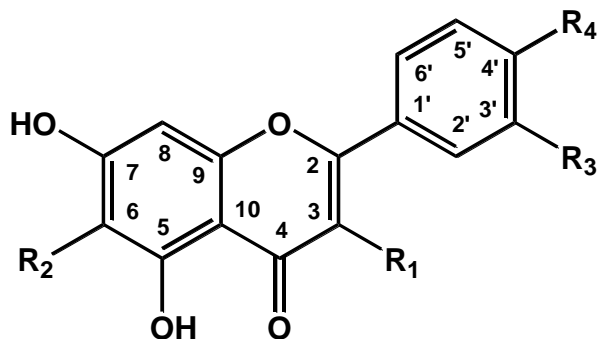
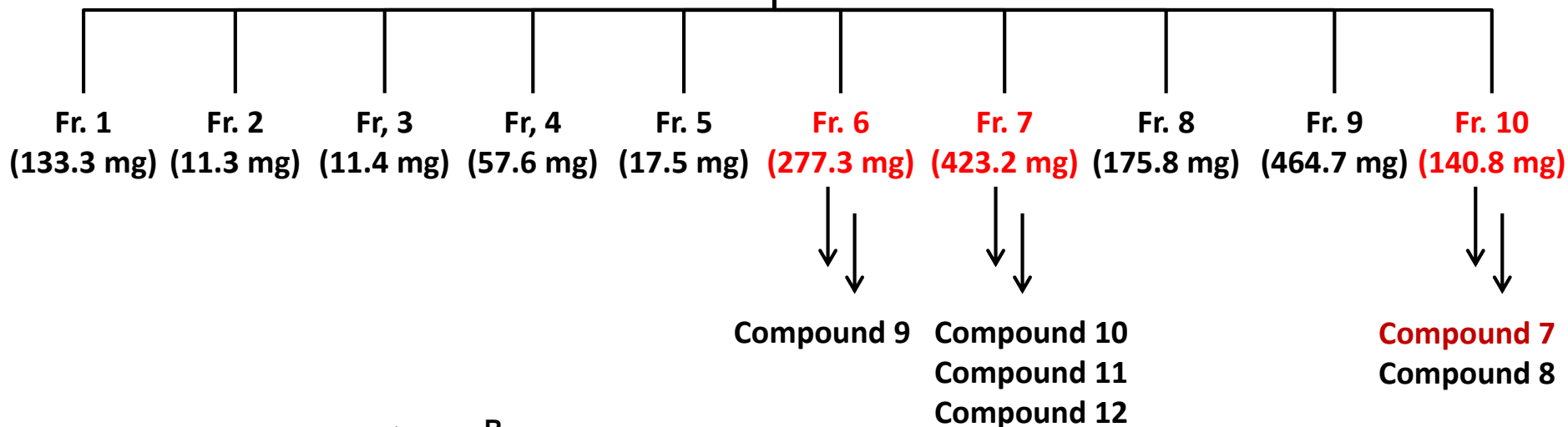
**12:** R<sup>1</sup>=R<sup>3</sup>=H, R<sup>2</sup>=OCH<sub>3</sub>, R<sup>4</sup>=OH



# Isolation of constituents, and determination of the structures of the isolated compounds

Bahia propolis  
Ethanolic extracts (1.92 g)

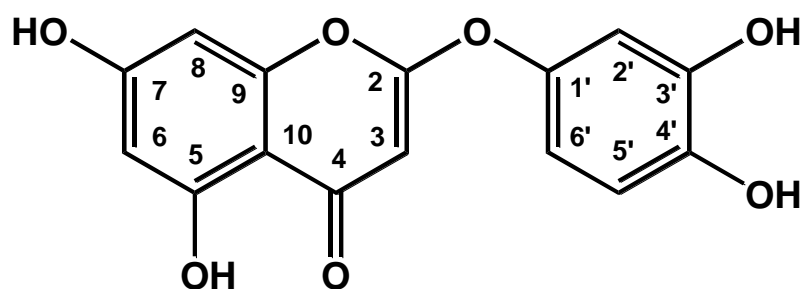
Silica gel column chromatography  
Gradient mixture of  $\text{CHCl}_3$ - $\text{CH}_3\text{OH}$



- Compound 8 :  $\text{R}^1=\text{OCH}_3$ ,  $\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{R}^4=\text{OH}$  ; 3-*O*-methylquercetin  
 Compound 9 :  $\text{R}^1=\text{R}^2=\text{R}^4=\text{OCH}_3$ ,  $\text{R}^3=\text{H}$  ; 3,6,4'-trimethoxychrysin  
 Compound 10 :  $\text{R}^1=\text{R}^2=\text{OCH}_3$ ,  $\text{R}^3=\text{H}$ ,  $\text{R}^4=\text{OH}$  ; 3,6-dimethoxyapigenin  
 Compound 11 :  $\text{R}^1=\text{R}^4=\text{OH}$ ,  $\text{R}^2=\text{OCH}_3$ ,  $\text{R}^3=\text{H}$  ; 6-methoxykaempferol  
 Compound 12 :  $\text{R}^1=\text{R}^3=\text{H}$ ,  $\text{R}^2=\text{OCH}_3$ ,  $\text{R}^4=\text{OH}$  ; 6-methoxyapigenin

\* Luteolin ;  $\text{R}_1=\text{R}_2=\text{H}$ ,  $\text{R}_3=\text{R}_4=\text{OH}$

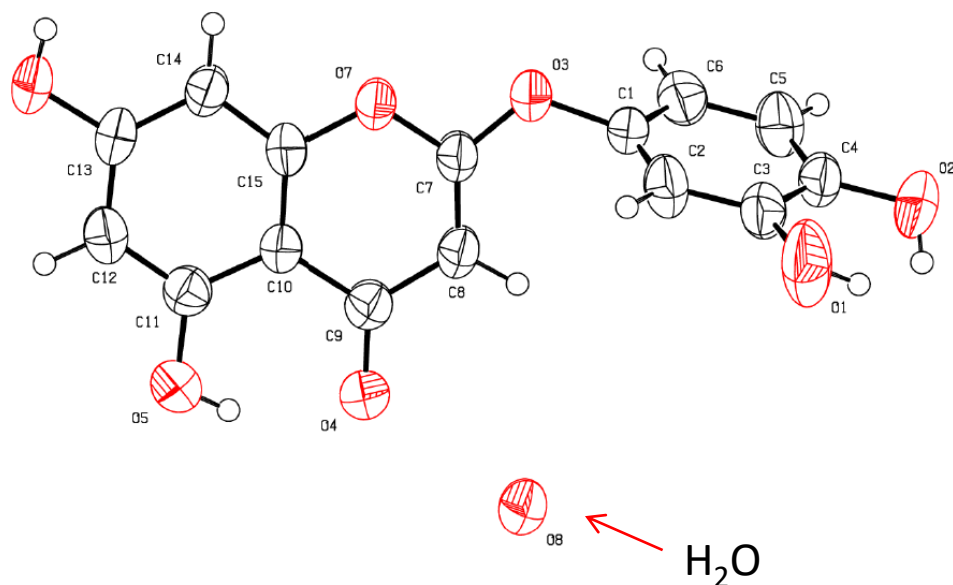
# The chemical structure of the novel isolated compound 7



**Compound 7**

(CCDC deposit No. 1532831)

ORTEP drawing of compound 7



T. Mitsui *et al.*, *Biosci. Biotech. and Biochem.*, 82(3), 417-421 (2018)

- Compound 7 is a **novel 2-phenoxychromone**.
- This is the first report on the discovery of 2-phenoxychromone from propolis.



# Anti-tumor activities of the isolated compounds

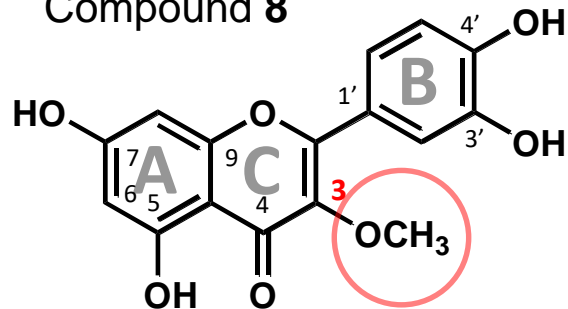
Compounds	IC <sub>50</sub> (μM)		
	MCF-7 (breast)	DLD-1 (colon)	A549 (lung)
<b>Novel 2-phenoxychromone (7)</b>	<b>174.4</b>	<b>65.8</b>	<b>81.9</b>
3-O-methylquercetin (8)	16.7	16.2	34.2
3,6,4'-trimethoxychrysin (9)	50.5	17.1	19.9
3,6-dimethoxyapigenin (10)	41.9	31.0	47.0
6-methoxykaempferol (11)	> 200.0	101.8	125.1
6-methoxyapigenin (12)	> 200.0	> 200.0	> 200.0

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- Anti-tumor activities were in the following order: compound 8 ≥ compound 9 ≥ compound 10 > compound 7 > compound 11 >>> compound 12.

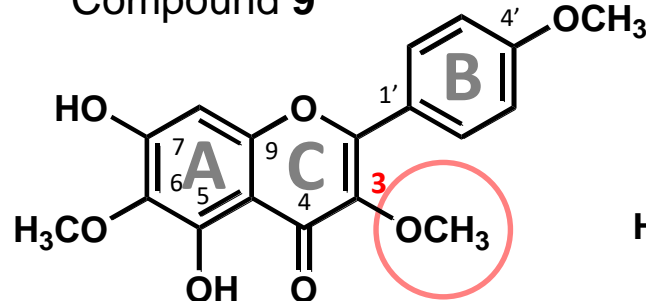
# Structure-activity relationship

Compound 8



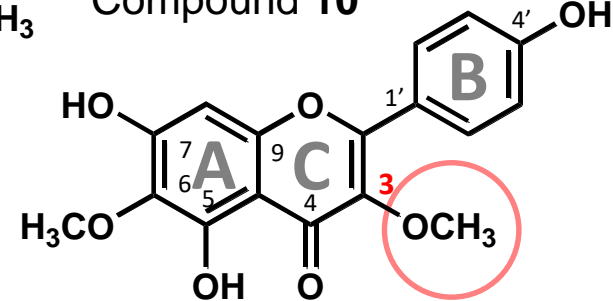
3-O-methylquercetin

Compound 9



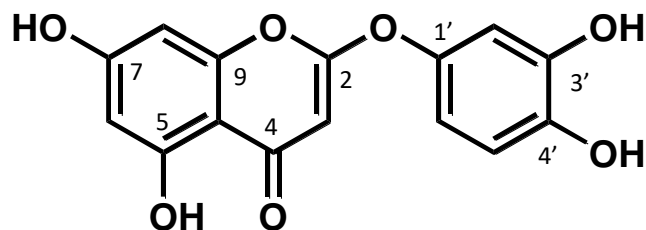
3, 6, 4'-trimethoxychrysin

Compound 10



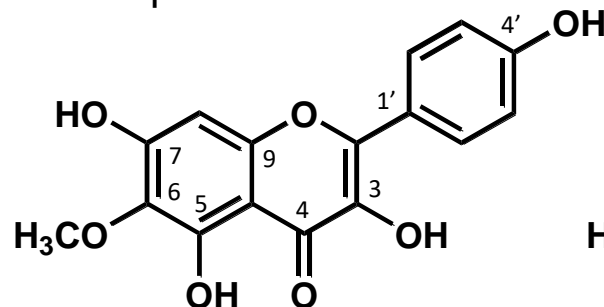
3, 6-dimethoxyapigenin

**Compound 7**



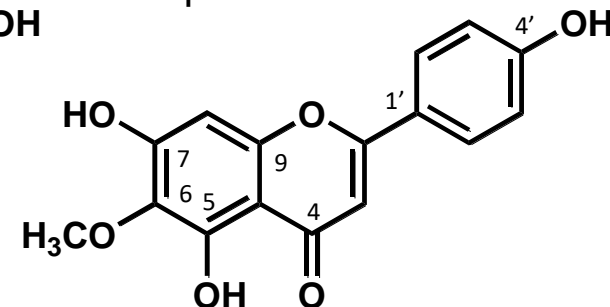
**2-phenoxychromone**

Compound 11



6-methoxykaempferol

Compound 12



6-methoxyapigenin

# Summary and Conclusion

## Parana propolis

- An ethanolic extract inhibited human tumor cell growth *in vitro*.
- Diterpenes, including a novel clerodane-type diterpene, were isolated in the extract.
- The isolated diterpenes exhibit the *in vitro* anti-tumor activity, and especially, the novel diterpene was the most potent.

## Bahia propolis

- An ethanolic extract inhibited human tumor cell growth *in vitro*.
- Flavones and a novel compound, 2-phenoxychromone, were isolated in the extract.
- The isolated flavones and 2-phenoxychromone exhibit the *in vitro* anti-tumor activity, and the 3-methoxy substituent of the C-ring may play an important role in this activity of flavones.

**In conclusion**, Brazilian propolis could be a potential target for natural products with anti-tumor activity.

# Acknowledgement

**Propolis from the state of Bahia was kindly provided by Mr. Kazushige Abe (Nordeste Co., Ltd., Japan).**

**We are grateful to Prof. Masahiro Ebihara (Gifu University, Japan) for conducting single crystal X-ray analysis.**

# Thank you for your attention



**Nagaragawa Research Center, API Co., Ltd., 692-3,  
Nagara, Gifu, Japan**

**URL: <http://www.api3838.co.jp/en/index.html>**