

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

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

Agenda

1. Introduction

2. Propolis from the state of Parana

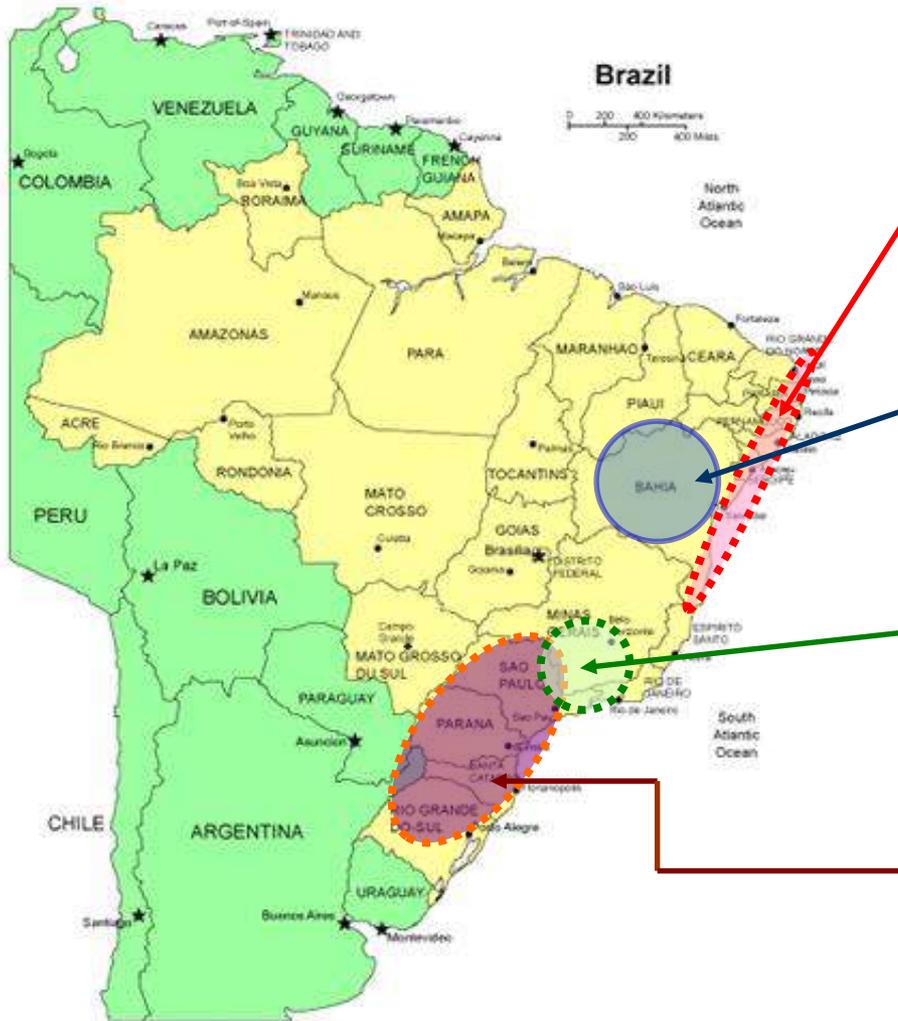
- Constituents
- *in vitro* anti-tumor activity

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 km²

Romania
238,397 km²

Brown Propolis
Parana



Analysis of Parana Propolis



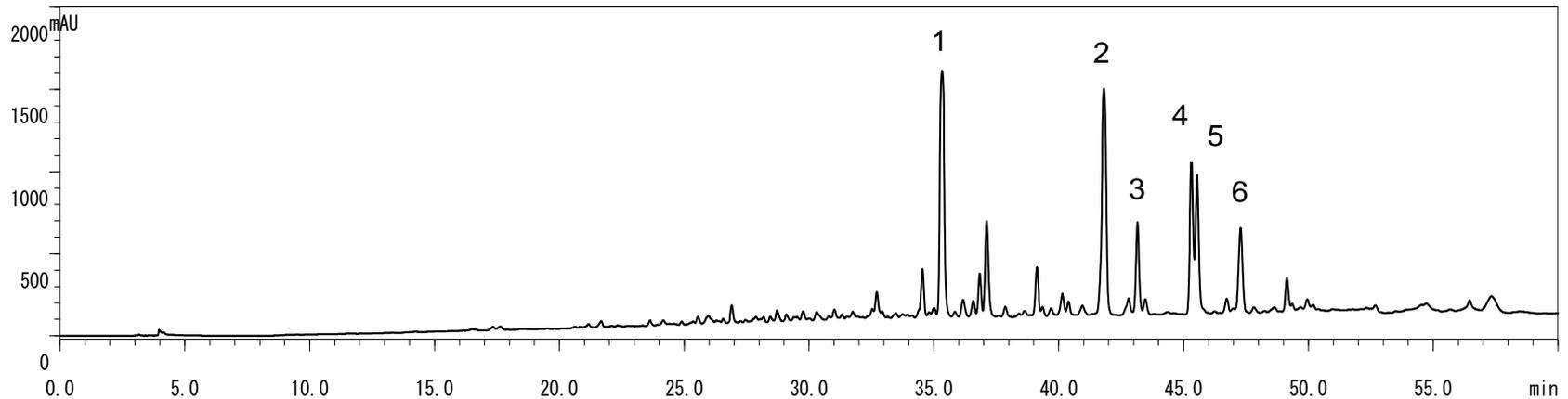
Extracts with 95% ethanol

《HPLC》

**Solvent: Gradient mixture of Acetonitrile-H₂O
(0.1% Phosphoric acid)**

Flow: 1.0 mL/min, Detect: 205 nm

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

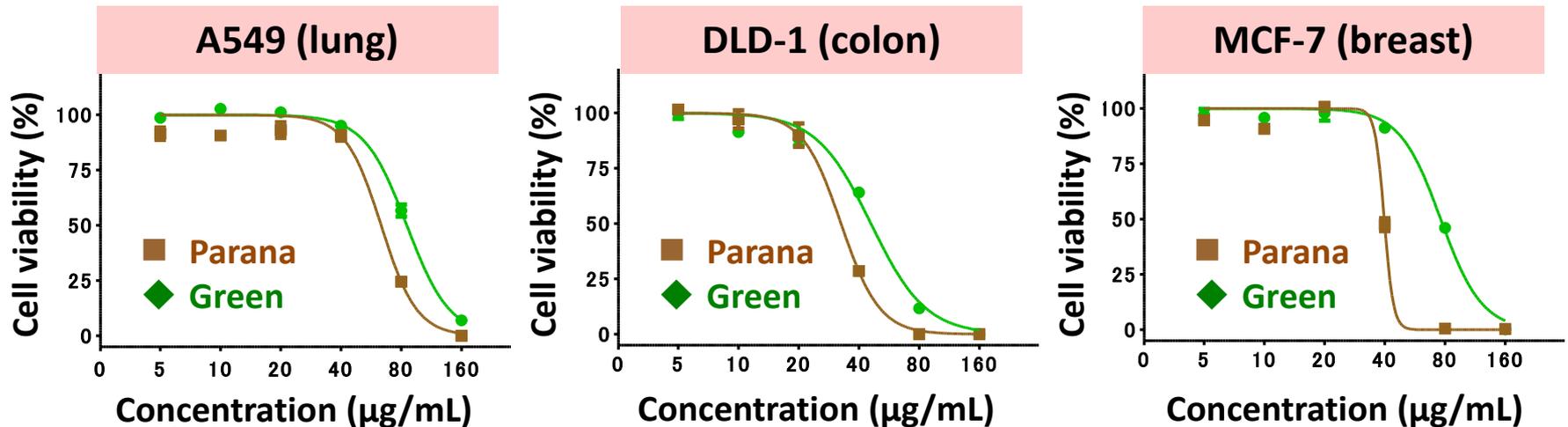


S. Tazawa et al., Natural Product Communications, 11(2), 201-205 (2016)

Anti-tumor activities of the ethanolic extracts



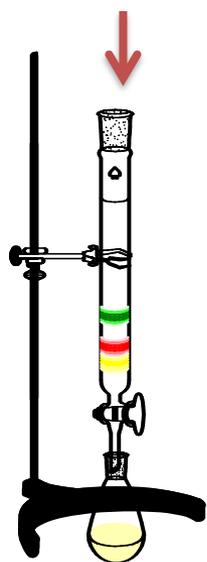
The tumor cells were cultured and then were treated with the extracts for 24 h. Growth inhibition was evaluated by quantifying living cells using the Cell Counting Kit-8 , and IC₅₀ values were determined in all tumor cell lines tested.



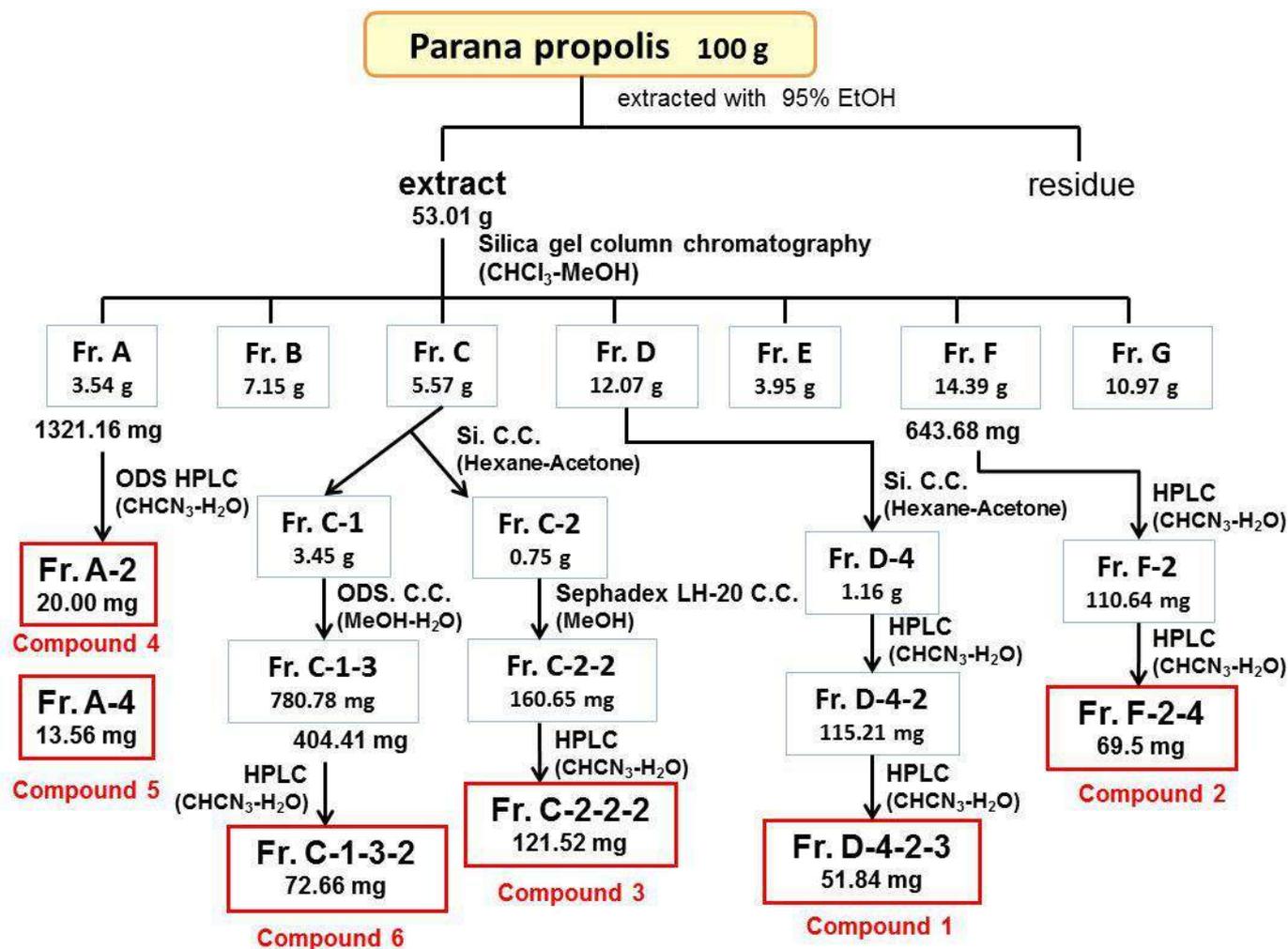
IC ₅₀ (µg/mL)	A549 (lung)	DLD-1 (colon)	MCF-7 (breast)
Parana Propolis	62.6	33.3	39.3
Green Propolis	85.6	42.8	77.6

Isolation of constituents

Ethanollic Extracts of Parana Propolis



Chromatographically separation



Determination of the structures of the isolated compounds

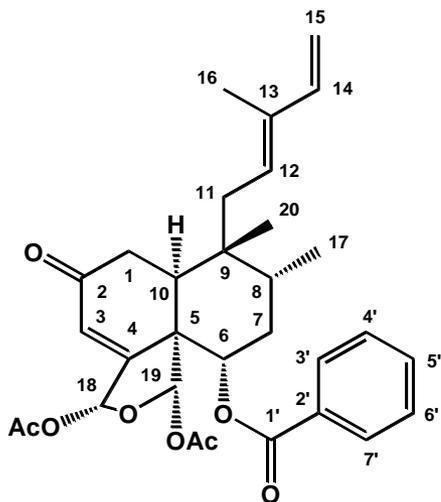
IR, UV
Functional group



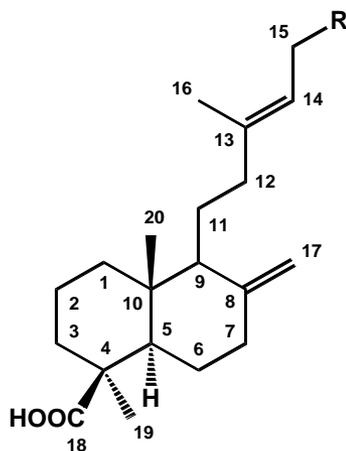
(HR) ESI-MS
Molecular Weight



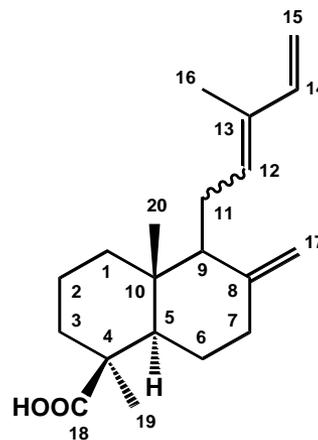
1D & 2D-NMR
Chemical Structure



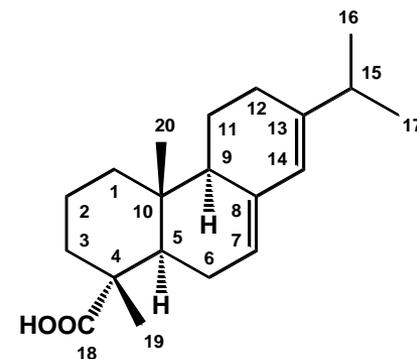
Novel compound 1



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 ₅₀ (μM)			
	LNCaP (prostate)	MCF-7 (breast)	DLD-1 (colon)	A549 (lung)
Novel clerodane diterpene (1)	6.2	6.3	6.8	12.7
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

Article of Newspaper (in Brazil, 1998)

"Propolis prevents the human body from invasion of tumors"

SAÚDE Pesquisa mostra que substância produzida pelas abelhas impede o crescimento de células cancerígenas

Própolis pode proteger corpo de tumores

Como foi feito o estudo

Foram coletadas cerca de 400 amostras de própolis de 12 tipos diferentes de regiões do país. As amostras foram analisadas em laboratório e os resultados foram publicados em uma revista científica.

Própolis

A substância produzida pelas abelhas para proteger a colônia de invasores, também pode ter propriedades que protegem o corpo humano de "invasões".

Um estudo realizado no Brasil recentemente demonstrou que diferentes tipos de própolis tinham diferentes efeitos de células cancerígenas em laboratório. O pesquisador Yang Kun Park, professor titular da Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas, realizou o estudo.

Ele havia sido orientado em um projeto de pesquisa que analisava o efeito da própolis sobre células cancerígenas de células de teste. O professor tem estudado a ação da própolis desde 1991. Foi analisada 400 amostras

de um tipo de própolis de Bahia (BA-6) (veja quadro ao lado). O método de cálculo da inibição de tumores utilizado no estudo teve como base de comparação os resultados obtidos pela droga Etoposide, a mais forte existente no mercado para combater o câncer. Esse método foi desenvolvido pelo Instituto Nacional do Câncer dos EUA. Os resultados apresentados foram confirmados por pesquisadores da Universidade de Carolina do Norte (EUA).

Somente na região Sul, Park descobriu sete tipos diferentes de própolis. O país tem uma biodiversidade muito rica, o que explica as várias variedades de própolis. Em países europeus ou nos EUA, as regiões das abelhas são todas iguais. Em seu estudo, os 12 tipos de própolis foram coletados em contato com diferentes células cancerígenas — do intestino, rim, mama, nariz e fígado.

Após duas análises — sempre para que os efeitos se reproduzíssem e fossem —, vieram os resultados. Em diferentes tipos de células de teste, a própolis mostrou-se mais potente que a droga Etoposide, a mais forte contra o câncer existente no mercado.

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
Bagé	3	14	39	8	18
Peiotas	1	53	45	12	31
Pelotas	3	50	47	13	33

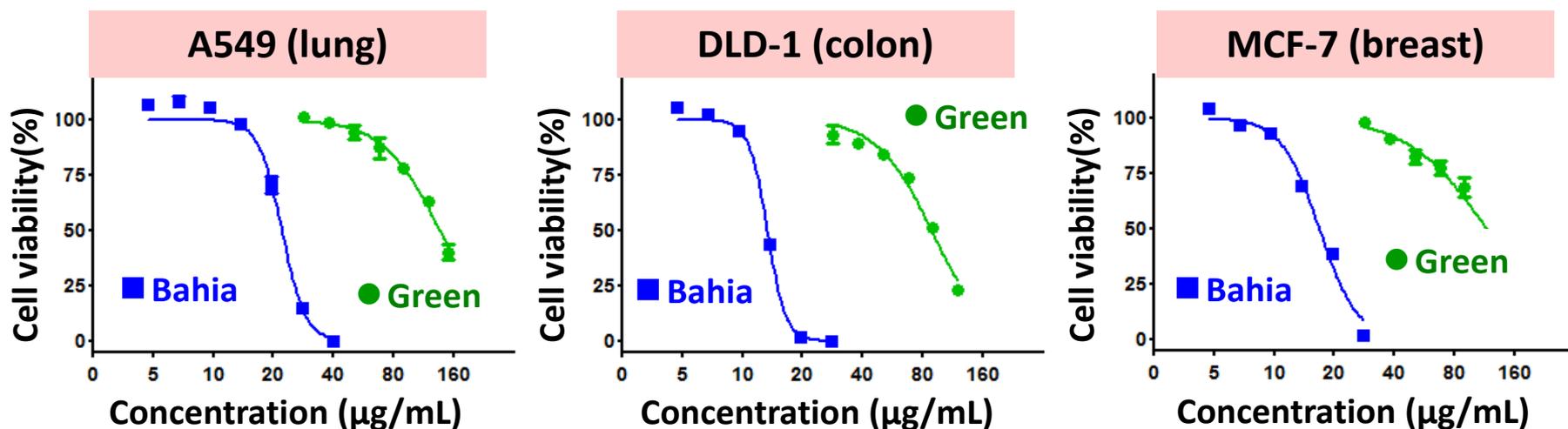
*O cálculo da porcentagem foi feito tomando-se como base a porcentagem de inibição da droga Etoposide, a mais forte contra o câncer existente no mercado. Esse é um método padrão de análise antitumoral, desenvolvido pelo Instituto Nacional do Câncer dos EUA.

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

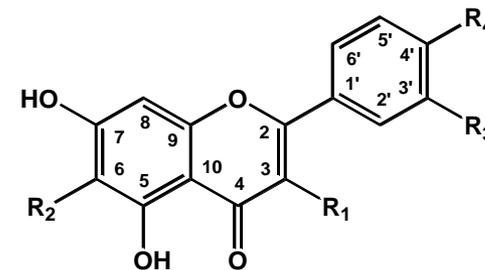
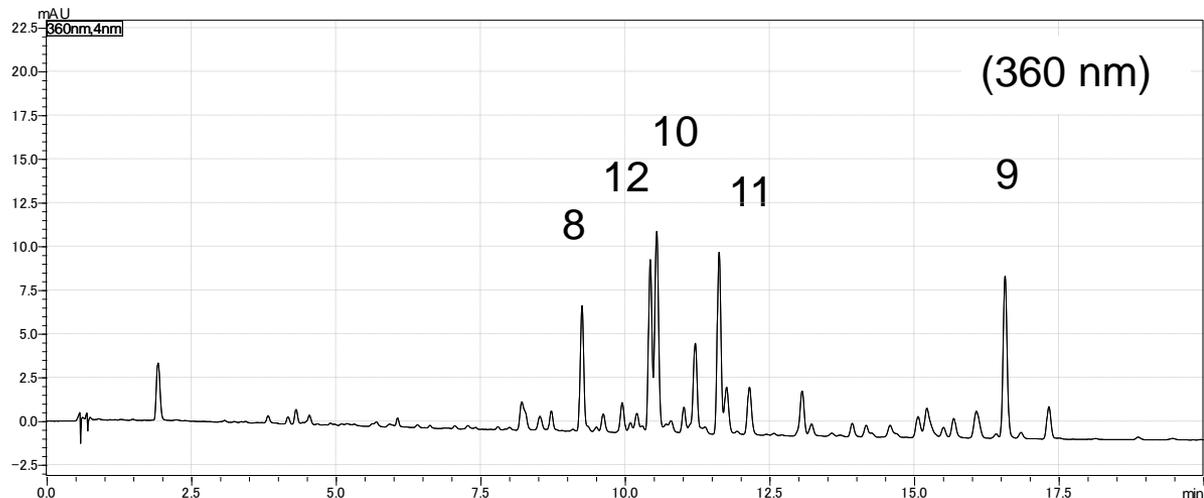
The tumor cells were cultured and then were treated with the extracts for 24 h. Growth inhibition was evaluated by quantifying living cells using the Cell Counting Kit-8, and IC₅₀ values were determined in all tumor cell lines tested.



IC ₅₀ (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



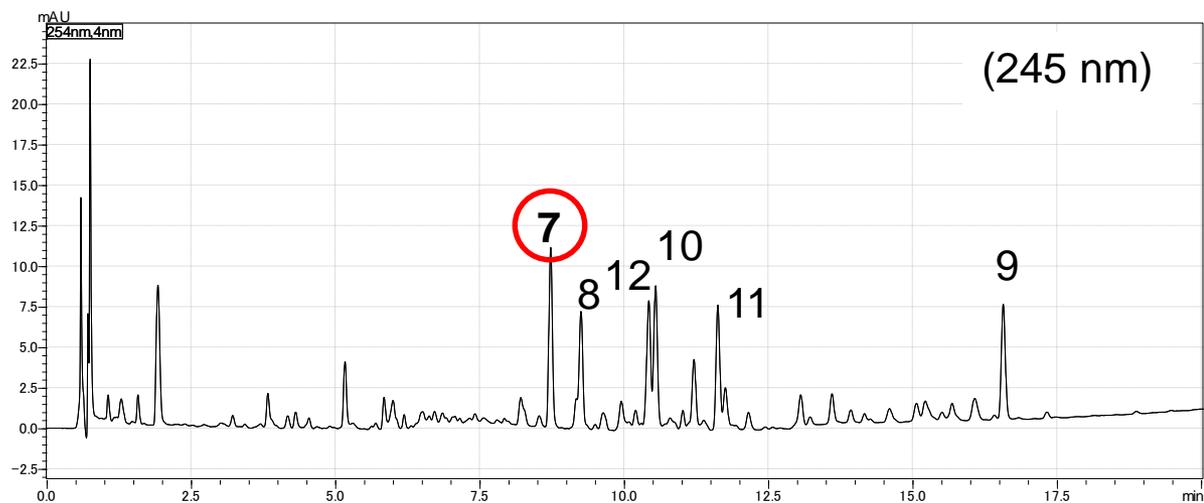
8: R¹=OCH₃, R²=H, R³=R⁴=OH

9: R¹=R²=R⁴=OCH₃, R³=H

10: R¹=R²=OCH₃, R³=H, R⁴=OH

11: R¹=R⁴=OH, R²=OCH₃, R³=H

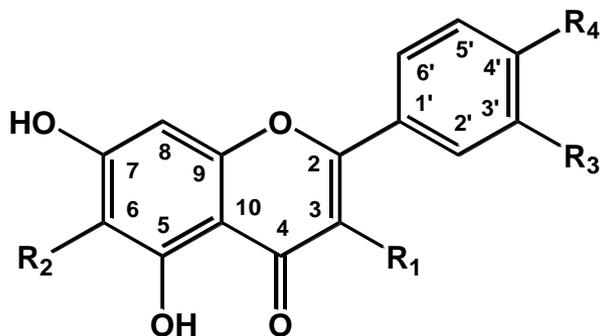
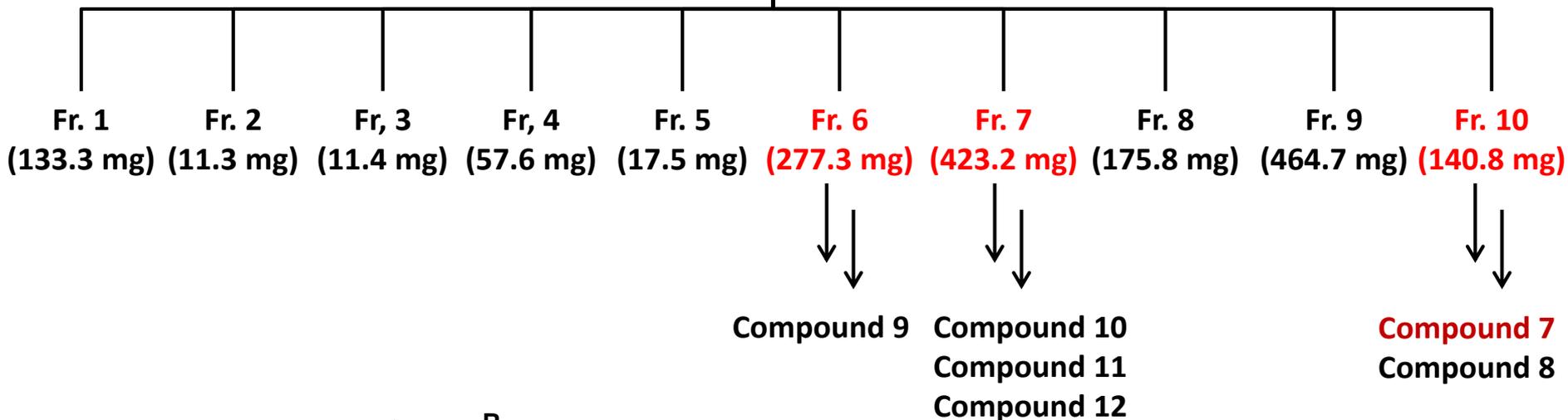
12: R¹=R³=H, R²=OCH₃, R⁴=OH



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

Bahia propolis
Ethanol extracts (1.92 g)

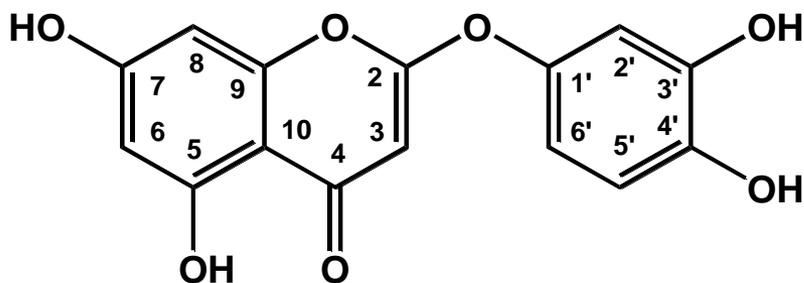
Silica gel column chromatography
Gradient mixture of CHCl₃-CH₃OH



- Compound 8 : R¹=OCH₃, R²=H, R³=R⁴=OH ; 3-*O*-methylquercetin
 Compound 9 : R¹=R²=R⁴=OCH₃, R³=H ; 3,6,4'-trimethoxychrysin
 Compound 10 : R¹=R²=OCH₃, R³=H, R⁴=OH ; 3,6-dimethoxyapigenin
 Compound 11 : R¹=R⁴=OH, R²=OCH₃, R³=H ; 6-methoxykaempferol
 Compound 12 : R¹=R³=H, R²=OCH₃, R⁴=OH ; 6-methoxyapigenin

* Luteolin ; R₁=R₂=H, R₃=R₄=OH

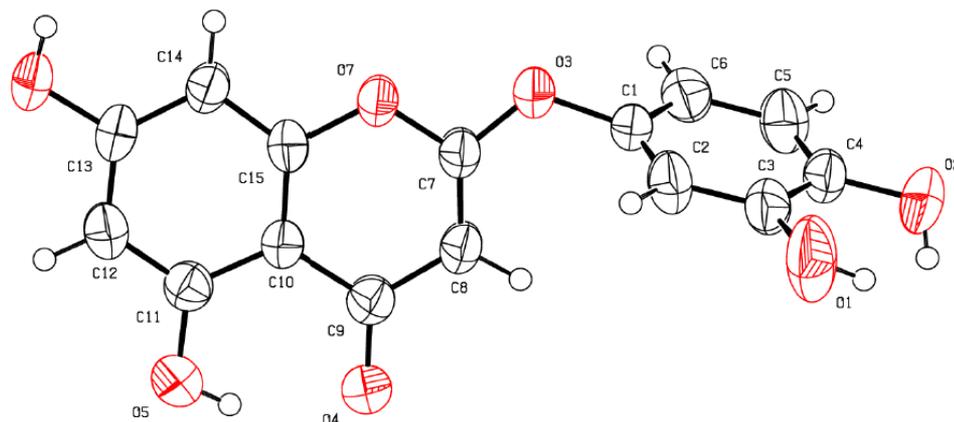
The chemical structure of the novel isolated compound 7



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

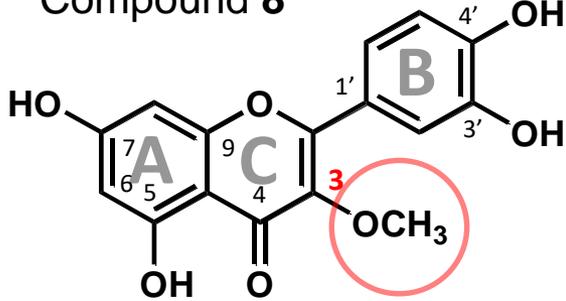
Compounds	IC ₅₀ (μM)		
	MCF-7 (breast)	DLD-1 (colon)	A549 (lung)
Novel 2-phenoxchromone (7)	174.4	65.8	81.9
3- <i>O</i> -methylquercetin (8)	16.7	16.2	34.2
3,6,4'-trimethoxychrysin (9)	50.5	17.1	19.9
3,6-dimethoxyapigenin (10)	41.9	31.0	47.0
6-methoxykaempferol (11)	> 200.0	101.8	125.1
6-methoxyapigenin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

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

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

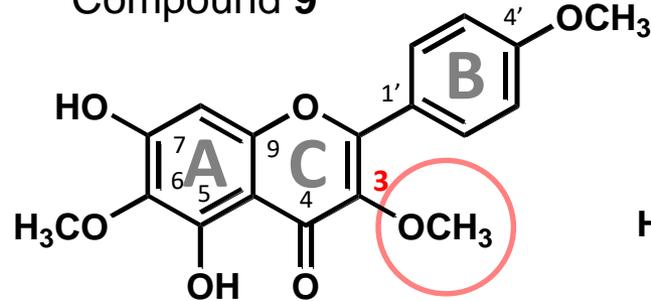
Structure-activity relationship

Compound 8



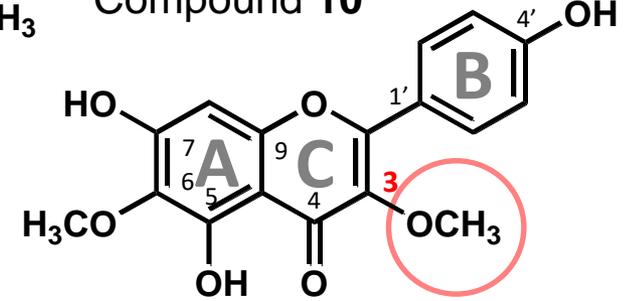
3-O-methylquercetin

Compound 9



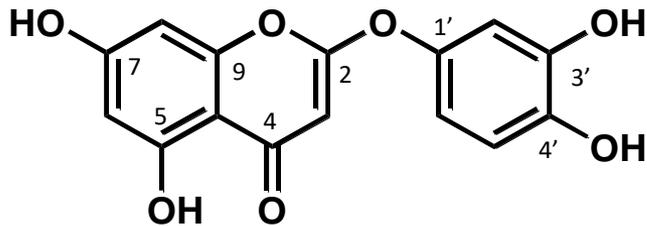
3, 6, 4'-trimethoxychrysin

Compound 10



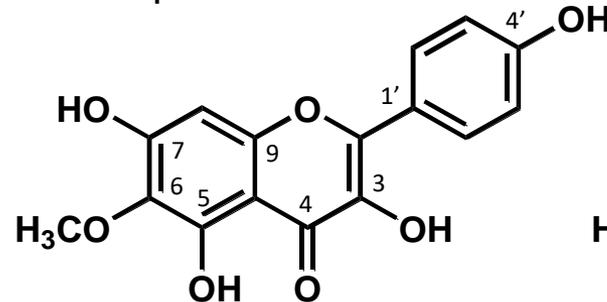
3, 6-dimethoxyapigenin

Compound 7



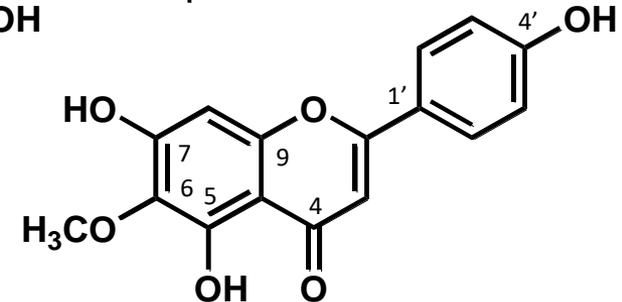
2-phenoxychromone

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



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