เอกสารอ้างอิง

1. Arimoto-Kobayashi S, Machida M, Okamoto K, Yamaguchi A. Evaluation of photo-mutagenicity and photo-cytotoxicity of food coloring agents. Mutagenesis. 2005, 20: 229-33.

2. Bagchi D, Garg A, Krohn RL, Bagchi M, Bagchi DJ, Balmoori J, Stohs SJ, Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. General Pharmaco.,1998, 30:771-8.

3. Castillo J, Benavente-Garcia O, Lorente J, Alcarez M, Redondo A, Ortuno A, Del Rio JA. Antioxidant activity and radioprotective effects against chromosomal damage induced in vivo by X-ray of flavan-3-ols (procyanidins) from grape seeds (Vitis vinifera): comparative study versus other phenolic and organic compounds. J.Agri. Food Chem., 2000, 48: 1738-1745.

4. De Flora S., Ferguson LR, Overview of mechanisms of cancer chemopreventive agents. Mutation Res., 2005, 591): 8-15.

5. De M, Krishna De A, Baneerjee AB, Antimicrobial screening of some Indian spicies. Phytotherapy Res., 1999, 13: 616-618.

23. Ellman GL. Tissue sulfhydryl groups. Arch Biochem. Biophys., 1959, 82: 70-77.

6. Gu L, Kelm MA, Hammerstone JF, Zhang Z, Beecher G, Holden J, Haytowitz D, Prior RL. Liquid chromatographic/electrospray ionization mass spectrometric studies of procyanidins in food. J.Mass Spec., 2003, 38: 1272-1280.

7. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 1974, 249: 7130-7139.2. Kelloff, GJ 2000. Perspectives on cancer chemoprevention research and drug development. Adv. Cancer Res. 78: 199-334.

8. Ito N, Tamano S. and Shirai T. A medium-term rat liver bioassay for rapid in vivo detection of carcinogenic potential of chemicals. Cancer Sci. 2003, Jan; 94(1):3-8.

9. Ito N, Tatematsu M, Hasegawa R, Tsuda H, Medium-term bioassay system for detection of carcinogens and modifiers of hepatocarcinogenesis utilizing the GST-P positive liver cell focus as an end point marker. Toxicol. Patho. 1989, 17: 630-641.

10. Klaunig JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol Toxicol. 2004, 44: 239-67

11. Komutarin T, Azadi S, Butterworth L, Keil D, Chitsomboon B, Suttajit M, Meade BJ. Extract of the seed coat of Tamarindus indica inhibits nitric oxide production by murine macrophages in vitro and in vivo. Food Chem Toxicol. 2004, 42: 649-58.

12. Matti R, Das UK, Ghosh D. Attenuation of hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats by aqueous extract of seed of tamarindus indica. Biol Pharm Bull., 2005, 28: 1172-1176.

13. Nakae D, Kobayashi Y, Akai H, Andoh K, Satoh H, Ohashi K, Tsutsumi M, Konishi Y, Involvement of 8-hydroxyguanine formation in the initiation of rat liver carcinogenesis by low dose levels of N-diethylnitrosamine. Cancer Res., 1997, 57, 1281-1287.

14. Ogiso T, Tatematsu M, Tamano S, Hasegawa R, Ito N, Correlation between medium-term liver bioassay system data and results of long-term testing in rats. Carcinogenesis, 1990, 11: 561-566.

15. Puatanachokchai R, Morimura K, Wanibuchi H, Oka M, Kinoshita A, Mitsuru F, Yamaguchi S, Funae Y, Fukushima S. Alpha-benzene hexachloride exerts hormesis in preneoplastic lesion formation of rat hepatocarcinogenesis with the possible role for hepatic detoxifying enzymes. Cancer Lett. 2005 Oct 21; [Epub ahead of print]

16. Pumthong G, Antioxidant activity of polyphenolic compounds extracted from seed coat of Tamarind indica Linn., Master degree thesis, Chiang Mai University, Thailand.

17. Ray RD, Parikh H and Bagchi D, Proanthocyanidin exposure to B6C3F1 mice significantly attenuates dimethylnitrosamine-induced liver tumor inducation and mortality by differentially modulating programmed and unprogrammed cell deaths. Mutation Res, 2005, Sep26(Epub ahead of print)

18. Rotruck JT, Pope AL, Gauther HE, Swanson AB, Hafeman DG, Hoekstna WG. Selenium: biochemical roles as a component of glutathione peroxidase. Science, 1973, 179: 588-590.

19. Sriplung H, Sontipong S, Martin N, Wiangnon S, Vootiprux V, Cheirsilpa A, Kanchanabat C, Khuhaprema, Cancer Incidence in Thailand, 1995-1997. Asian Pacific J Cancer Prev, 2005, 6: 276-281.

20. Sudjaroen Y, Haubner R, Wurtele G, Hull WE, Erben G, Spiegelhalder B, Changbumrung S, Bartsch H, Owen RW. Isolation and structure elucidation of phenolic

antioxidants from Tamarind (*Tamarindus indica L.*) seeds and pericarp. Food Chem Toxicol. 2005, 43: 1673-82.

21. Yamakoshi J et al, Safety evaluation of proanthocyanidin-rich extract from grape seeds. Food Chem Toxicol, 2002, 40: 599-607.

22. Yu C.L. and Swaminathan B., Mutagenicity of proanthocyanidins. Food Chem Toxicol, 1987, 25:135-9.

23. Zhang XY et al, Proanthocyanidin from grape seeds enhances anti-tumor effect of doxorubicin both in vitro and in vivo. Pharmazie, 2005, 60: 533-8.

ภาคผนวก

การศึกษาสารฟีนอลิกในสารสกัดหยาบจากเปลือกหุ้มเมล็ดมะขามด้วยวิธี Liquid chromatography-Electrospray ionization mass spectrometry โดยรศ.ดร.สุกัญญา วงศ์พรชัย ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่

HPLC condition:

Column LC column, Zorbax Eclipse-C18 (3.5 μm, 4 × 100 mm), Agilent Technologies, USA

Mobile phase A: Methanol; B: 0.5% Acetic acid in water

Gradient profile:	0	min	95	% B
	0-100	min	0	% B
	100-120	min	0	% B
Flow rate	0.2 ml/min			

MS condition:

Ion Source (Electrospray Ionization)

Capillary (kV)	3.0 k
Cone (ev)	30(ev)
Extractor (V)	10 V
RF Lens (V)	0.5 V
Source Temperature (^{0}C)	100 ^{0}C
Desolvation Temperature (^{0}C)	200 ^{0}C

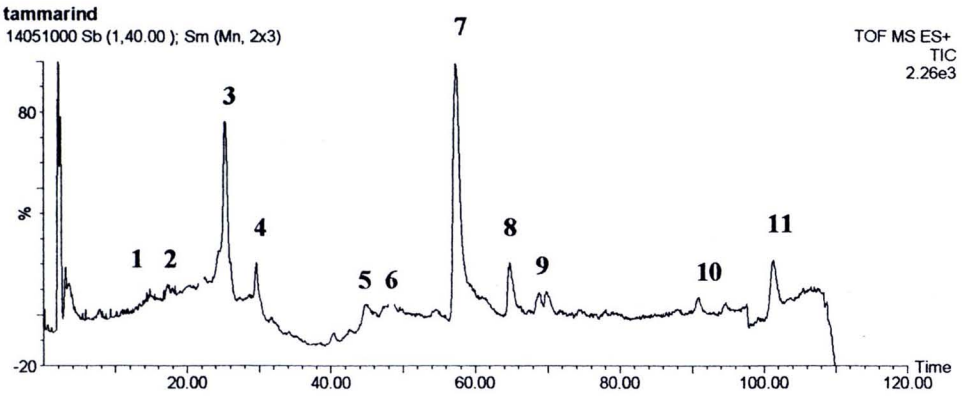
MS 1 (Quadrupole)

LM Resolution	5.0
HM Resolution	5.0
Collision Energy (eV)	10.0 eV
Ion Energy (V)	2.0 V
Steering (V)	2.0 V
Entrance (eV)	65.0 eV
Pre-filter (V)	5.0 V

MS 2 (Time-of-Fight)

Transport (V)	4.0
Aperture 2 (V)	14.6
Acceleration (V)	200
Focus (V)	0
Tube Lens	75
Offset 1, Offset 2	-0.1, 0.0
Pusher (V)	980
TOF (kV)	9.1
Reflectron	35.25
Pusher Cycle Time (µs)	Auto
Pusher Frequency (Hz)	16129.03
Multiplier (V)	650
MCP (V)	2000

ผลการวิเคราะห์ พบว่าสารสกัดหยาบจากเปลือกหุ้มเมล็ดมะขามด้วยเมธานอล ประกอบด้วย Procyanidin dimer 78.12%, Procyanidin trimer 19.64% และ Procyanidin tetramer 0.04% ดังรูปและตาราง สำหรับ Peak 11 คาดว่าเป็น contaminate จากพลาสติก

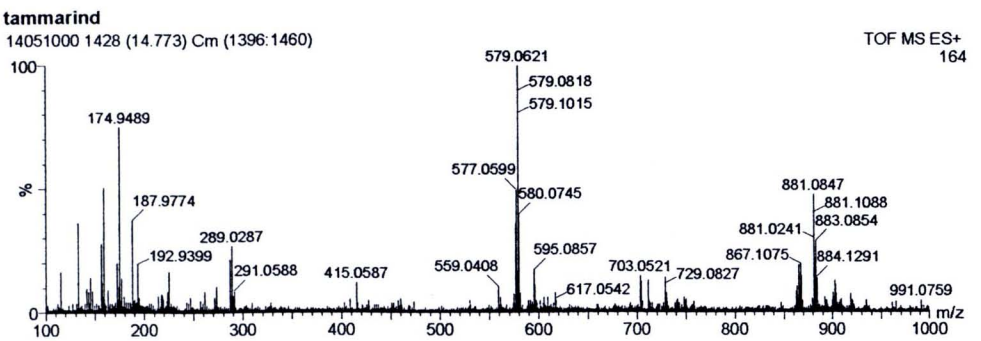


LC-MS chromatogram of the Tamarind extract obtained by LC-MS

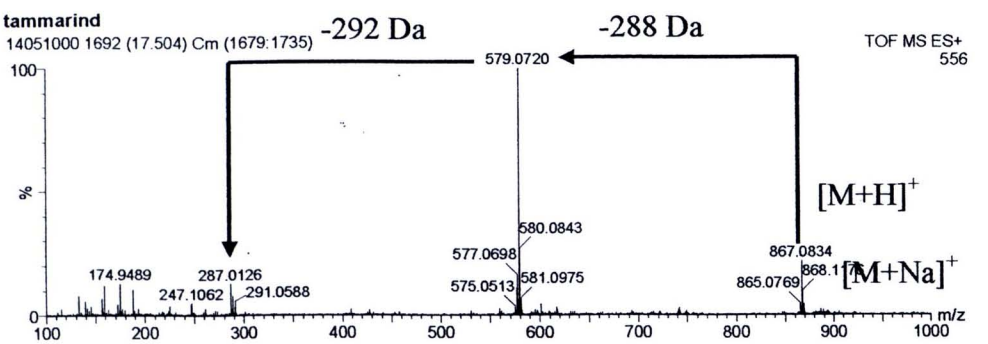
Identified Compounds	Peak number	Retention time [min]	Peak area	% Relative abundance of compounds	% Relative abundance of each group
Procyanidin dimer	3	25.24	1452.35	28.56	78.1172
	7	57.42	2302.29	45.27	
	9	68.8	109.05	2.14	
	10	87.77	109.31	2.15	
Procyanidin trimer	1	14.77	76.86	1.51	19.6443
	2	17.5	106.18	2.09	
	4	29.6	225.22	4.43	
	5	45.06	234.50	4.61	
	8	64.74	356.34	7.01	
Procyanidin tetramer	6	48.31	113.85	2.24	0.0440

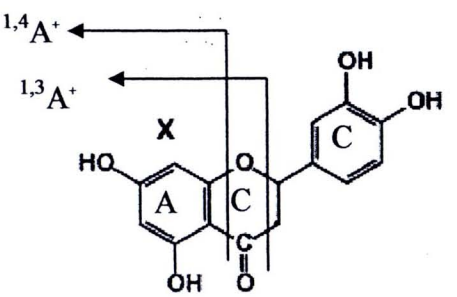
ผลการวิเคราะห์โครงสร้างของแต่ละ Peak ด้วย Mass Spectrometry

Peak number 1; Retention time 14.77 min

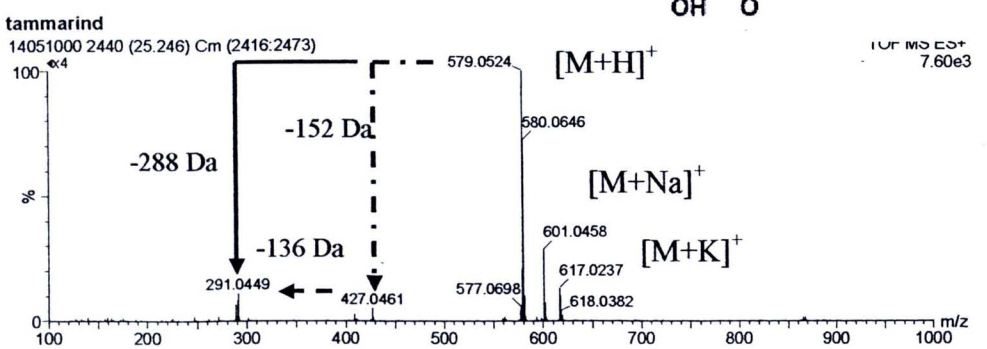


Peak number 2; Retention time 17.50 min

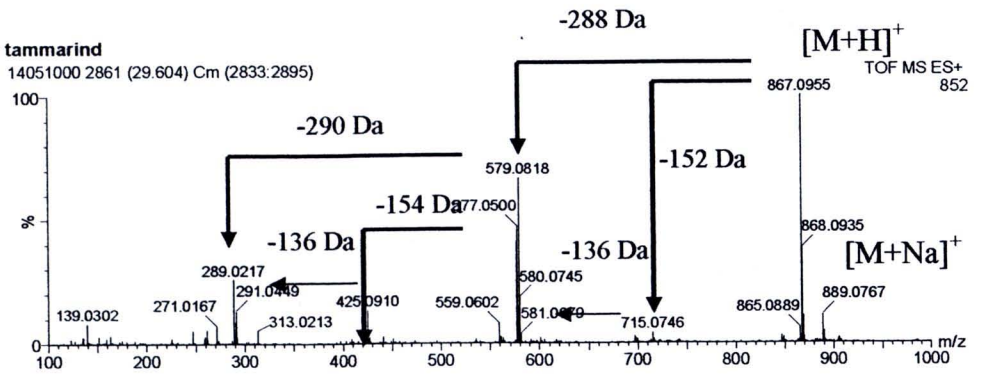




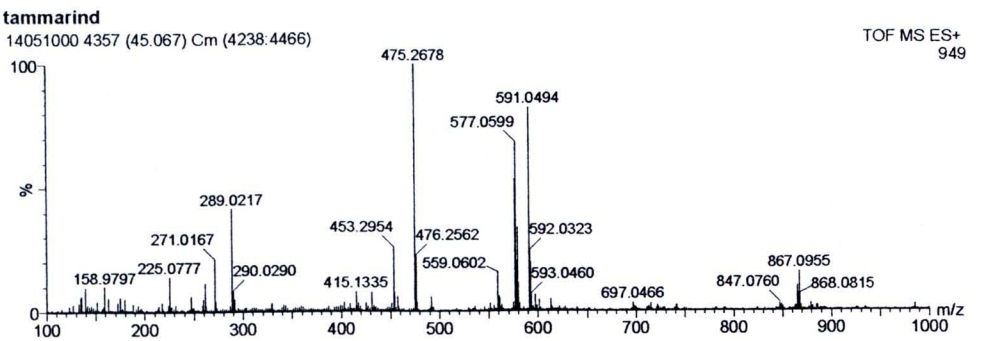
Peak number 3; Retention time 25.24 min



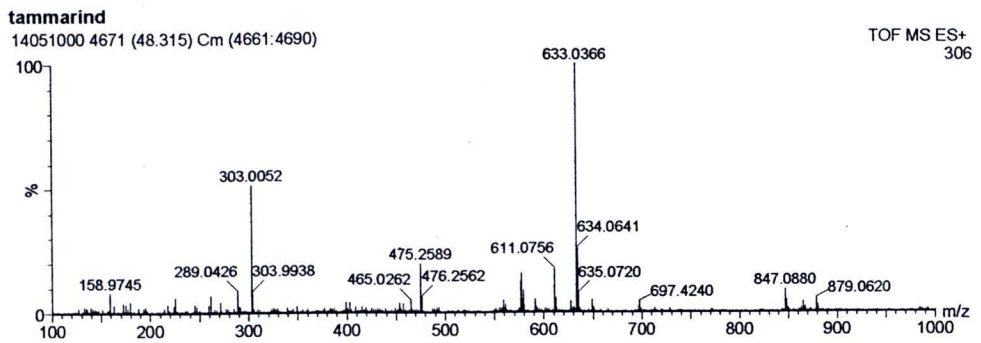
Peak number 4; Retention time 29.60 min



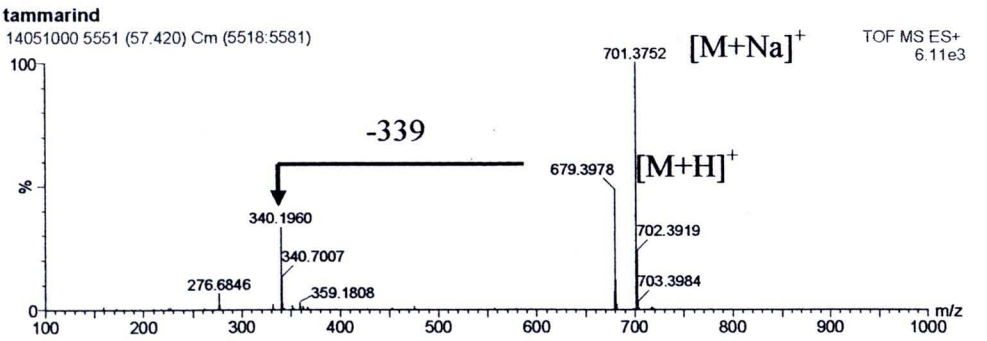
Peak number 5; Retention time 45.06 min



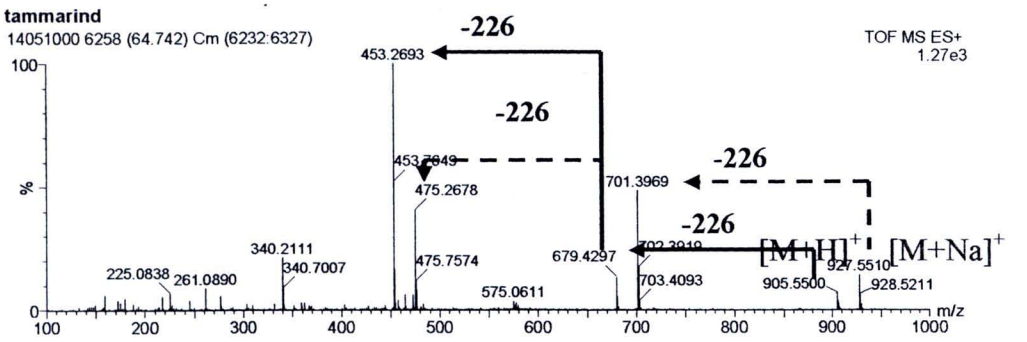
Peak number 6; Retention time 48.31 min



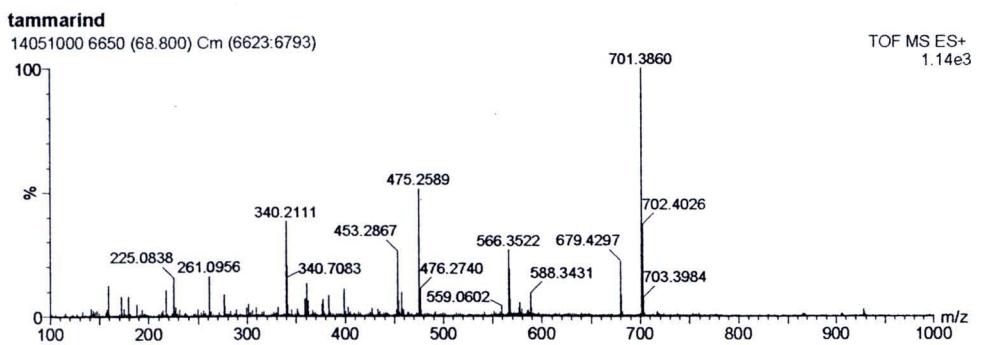
Peak number 7; Retention time 57.42 min



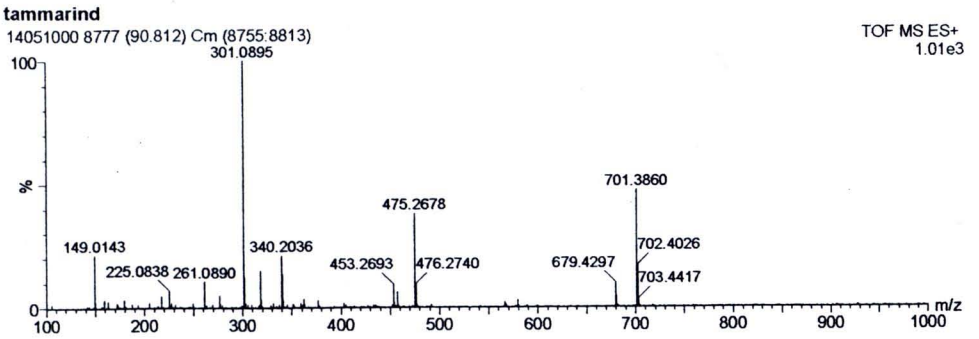
Peak number 8; Retention time 64.74 min (น่าจะเป็น Triimer ของ ฟลาโวนอยด์)



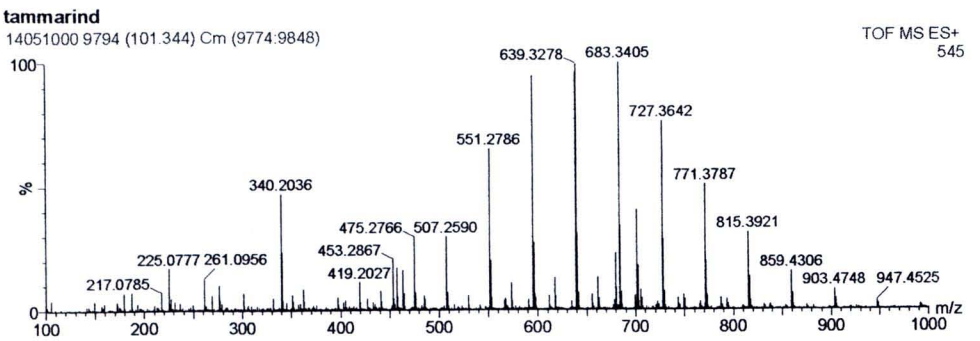
Peak number 9; Retention time 68.80 min

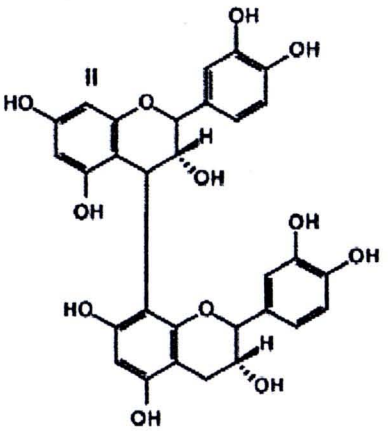


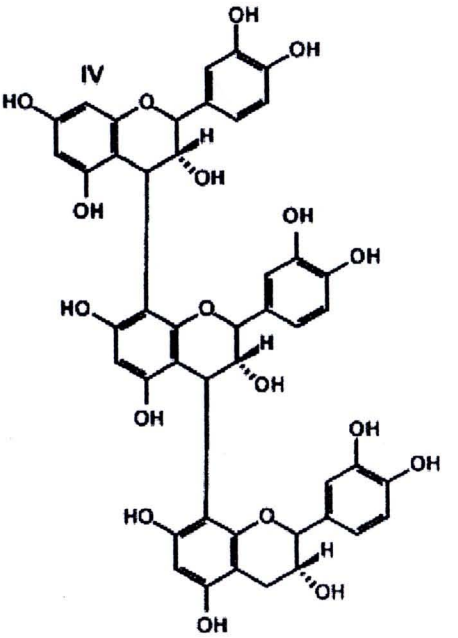
Peak number 10; Retention time 87.77 min

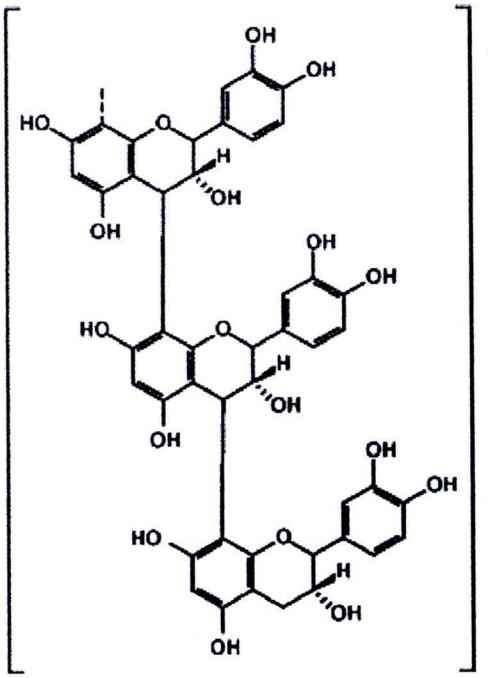


Peak number 11; Retention time 101.34 min









V-VII = 866 + 288n

The structures of the oligomeric flavonoids are procyanidin B_2 (II), procyanidin trimer (IV), procyanidin tetramer (V), procyanidin pentamer (VI) and procyanidin hexamer (VII)

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

Wongpoomchai R., Charoensin S., Suttajit M., Wanibuchi H. and Fukushima S. Chemopreventive effects of Tamarind (*Tamarindus indica* L) husk extract on the early stages of rat hepatocarcinogenesis. กำลังเตรียม Manuscript เพื่อลงตีพิมพ์

2. การนำผลงานวิจัยไปใช้ประโยชน์

-เชิงวิชาการ :

-ใช้องค์ความรู้ที่ได้จากงานวิจัยนี้ในการเรียนการสอนนักศึกษาระดับปริญญาตรีและบัณฑิตศึกษาของมหาวิทยาลัยเชียงใหม่

-จากการพัฒนาโมเดลทดสอบในหนูทดลองนี้ที่ไม่เคยมีการวิจัยมาก่อนในประเทศไทย ทำให้ได้เทคนิคและวิธีในการทดสอบฤทธิ์ก่อมะเร็งและต้านมะเร็งตับของสมุนไพรไทยเพิ่มขึ้น และใช้เป็นส่วนหนึ่งของวิทยานิพนธ์นักศึกษาระดับบัณฑิตศึกษา

-เชิงสาธารณะ:

-ทำให้มีเครือข่ายความร่วมมือกับ Department of Pathology, Graduate School of Medicine, Osaka City University เมืองโอซาก้า ประเทศญี่ปุ่น

-ทำให้มีความร่วมมือกับนักวิชาการที่ทำงานวิจัยทางด้านสมุนไพร โดยใช้โมเดลทดสอบฤทธิ์ก่อมะเร็งและต้านมะเร็งตับในหนูทดลองจากงานวิจัยนี้ในการขยายผลการทดลองที่ได้ทำมาก่อน เช่น ตำรับยาตรีผลาของกลุ่มนักวิจัยมหาวิทยาลัยธรรมศาสตร์

และ กระชายดำของกลุ่มนักวิจัยมหาวิทยาลัยขอนแก่น

3. การเสนอผลงานในที่ประชุมวิชาการ

-ต่างประเทศ

Wongpoomchai R., Charoensin S., Petra R., Wanibuchi H., Fukushima S., and Suttajit M. Chemopreventive effects of Tamarind (*Tamarindus indica* L) husk extract on the early stages of rat hepatocarcinogenesis. Oral presention in 1st Asian Conference on Environmental Mutagens. Kitakyushu, Japan. November 29- November 30, 2007.

-ในประเทศ

1. Wongpoomchai R., Dalai N., Suttajit M., Wanibuchi H., and Fukushima S. Chemopreventive effects of tamarind (*Tamarindus indica* L.) seed coat extract on the early stages of hepatocarcinogenesis in rat. Poster presention in The Second International Conference on Natural Products for Health and Beauty. Phayao, Thailand. December 17-19, 2008.

O-03 Assessment of polycyclic aromatic hydrocarbon residues and cytotoxicity of repeatedly used cooking oils

Supatra PORASUPHATANA, Jetana WEERAKUL, Pramote MAHAKUNAKORN, Wongwiwat TASSANEEYAKUL : *Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand*

Deep-frying cooking is popular in Thailand and the utilization of cooking oils is usually found to be abused. This practice may cause toxicity including mutagenesis and carcinogenesis due to oil deterioration. It is well established among international regulations on the recommendations and legal provisions of cooking oil quality control. One of the parameters which became the most accepted is the Total Polar Compounds (TPCs). In Thailand, the concentration of TPCs in repeatedly used cooking oils is legally limited at 25% of the sample. The regulation setting of %TPCs gives an approximate evaluation of the total degradation compounds in cooking oils, however, its correlation with cytotoxicity has not yet been addressed. The objective of this study was to assess the correlation between %TPCs of cooking oils (soybean oil (SBO) and palm oil (PO)) and their cytoxicities. Frying SBO and PO were prepared at high temperature (165oC). Oil samples were periodically collected and %TPCs were determined according to the standard IUPAC 2.507 method and then grouped according to %TPCs to be 0-8 (Gr. I), 9-16 (Gr. II), 17-24 (Gr. III) and 25-30 (Gr. IV). PAHs analysis was carried out with liquid extraction followed by gas chromatography (GC) using PAH Mixed consisted of 18 compounds as standards. Cytotoxicity was measured as cell viability and micronucleus assays. Results showed undetectable levels of PAHs in frying SBO and PO (Gr. I-III) whereas fluorene, phenanthrene and anthracene were detected in PO (Gr. IV), indicating the formation of PAHs in PO when its %TPCs is above the limitation of 25%. Cytotoxicities of extracts from repeatedly used cooking SBO and PO will be discussed.

Assessment of polycyclic aromatic hydrocarbon residues and cytotoxicity of repeatedly used cooking oils

PORASUPHATANA Supatra, WEERAKUL Jetana, MAHAKUNAKORN Pramote, TASSANEEYAKUL Wongwiwat : *Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand*

O-04 Chemopreventive effects of Tamarind (*Tamarindus indica* L) husk extract on the early stages of rat hepatocarcinogenesis

Rawiwan P WONGPOOMCHAI[1], Suphachai CHAROENSIN[1], Rampeung PETRA[1], Hideki WANIBUCHI[2], Shoji FUKUSHIMA[3], Maitree SUTTAJIT[4] : *Dept.Biochemistry, Fac.Medicine, Chiang Mai University, Chiang Mai, Thailand[1], Dept.Pathology, Osaka City University Medical School, Osaka, Japan[2], Japan Bioassay Research Center, Hadano, Kanagawa, Japan[3], Dept.Chemistry, School of Science and Technology, Naresuan University, Phayao Campus,Thailand[4]*

Tamarind (*Tamarindus indica* L) grows naturally in many tropical and subtropical regions including Thailand. It has been used as food seasoning and traditional medicines. High level of antioxidant compounds were found in its husk, a seed coat. The effects of tamarind husk extract (THE) on the early stages of rat hepatocarcinogenesis were investigated using medium-term rat liver bioassay, Ito's model. Male wistar rats were divided into 6 groups. Group 1 to 4 were intraperitoneally injected by diethylnitrosamine (DEN), a liver genotoxic carcinogen, at the first week of an experiment and group 5 to 6 were received saline injection. All rats were partially hepatectomized at the fourth week to amplify initiated cells. Group 2 and 3 were fed via intragastrium with THE at concentration of 20 and 100 mg/kg body weight, respectively, 5 days a week from the third week for 6 weeks. Group 4 and 6 were administered high dose of THE, 500 mg/kg body weight. Group 1 and 5 were received distilled water as control. All rats were sacrificed at the end of week 8. THE did not affect on body weight change, some vital organ weights and serum AST, ALT and ALP activities. It, however, attenuated hepatotoxicity in rat induced by DEN. THE did not influence on rat liver preneoplastic lesions, glutathione-*S*-transferase placental form (GST-P). It reduced number of GST-P positive foci in livers initiated by DEN. These results suggest inhibitory effect of tamarind husk extract on the early stages of hepatocarcinogenesis in rat after initiated by DEN. The mechanism of chemopreventive activity of tamarind husk extract is under investigated.

Chemopreventive effects of Tamarind (Tamarindus indica L) husk extract on the early stages of rat hepatocarcinogenesis

WONGPOOMCHAI Rawiwan P[1], et al.

1st Asian Conference on Environmental Mutagens
36th Annual Meeting of The Japanese Environmental Mutagen Society

Program・Abstracts

Thursday, November 29 – Friday, November 30, 2007

Venue: Kitakyushu International Conference Center
9-30, Asano, Kokurakita-ku, Kitakyushu 802-0001 Japan
TEL : 093-541-5931
(http://www.convention-a.jp/kokusai/)

General Secretariat : Dept. Environ. Oncology,
Institute of Industrial Ecological Sciences (IIES)
University of Occupational and Environmental Health, Japan (UOEH, Japan)
1-1, Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555 Japan
TEL : 093-691-7469 FAX : 093-601-2199
E-mail : acem2007@mbox.med.uoeh-u.ac.jp
URL : http://www.j-ems.org/anu_meeting/2007/index.html

Post A-3

Chemopreventive effects of tamarind (*Tamarindus indica* L.) seed coat extract on the early stages of hepatocarcinogenesis in rat

Wongpoomchai, R.[1], Dalai N.[1], Suttajit, M.[2] Wanibuchi, H.[3] and Fukushima, S.[4]
[1]Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200
[2] School of Science and Technology, Naresuan University, Phayao Campus, Phayao 56000, Thailand
[3]Department of Pathology, Graduate School of Medicine, Osaka City University, Osaka, Japan
[4]Japan Bioassay Research Center, Hadano, Kanagawa, Japan
E-mail:rawibiochem@yahoo.com

Objective: Tamarind (Tamarindus indica L.) has been used as food seasoning and traditional medicines. High level of antioxidant compounds were found in its husk. This study aimed to investigated inhibitory effects of tamarind seed coat extract (TSE) on the early stages of rat hepatocarcinogenesis.

Methods: Male wistar rats were divided into 6 groups. Group 1-4 were intraperitoneally injected by diethylnitrosamine (DEN), a genotoxic hepatocarcinogen, at the first week of an experiment and group 5 and 6 were received saline injection. All rats were partially hepatectomized at the fourth week to amplify initiated hepatocytes. Group 2 and 3 were fed with TSE at concentration of 20 and 100 mg/kg bw, respectively for 6 weeks. Group 4 and 6 were administered high dose of TSE. All rats were sacrificed at the end of week 8. Three to four mm thick sections from the right, medial and left lateral lobes were fixed in 10% buffered formalin and embedded in paraffin for routine histological and immunohistochemical analyses. The remaining liver tissue was frozen under liquid nitrogen and stored at -80°C until applied for biochemical analyses.

Results: TSE did not affect on body weight change, some vital organ weights and liver function test. TSE did not affect on preneoplastic lesions, glutathione-S-transferase placental form, in the rat livers.However, TSE at low concentration reduced both number and area of preneoplastic lesions in liver initiated by DEN. It also decreased number of PCNA, a cellular proliferation marker, and increased glutathione and GST activity in the rat liver treated with DEN. High concentration of tamarind husk extract did not show any protective effects. It might cause from high content of tannin which interfere the absorption and protein utilization in the body.

Conclusion: These results suggest inhibitory effect of tamarind husk extract on the early stages of hepatocarcinogenesis in rat after initiated by DEN. The possible mechanism might be due to enhancement detoxifying system and suppression of cellular proliferation in the rat liver.

Key Words: cancer chemoprevention, hepatocarcinogenesis, tamarind

ISSN 1906-214

NARESUAN PHAYAO Journal

An Academic Journal of Naresuan University at Phayao Volume 1 Supplement (1) 2008

PROGRAM AND ABSTRACTS

From

The Second International Conference on Natural Products for Health and Beauty (NATPRO)

17 – 19 December, 2008

Naresuan University at Phayao, Phayoa Province, Thailand

2nd NATPRO

Natural Products for Health and Beauty

Naresuan University at Phayao, Thailand

สำนักงานคณะกรรมการวิจัยแห่งชาติ ห้องสมุดงานวิจัย

Organized by Naresuan University, Thailand

Under the Auspices of

Higher Education Commission, Ministry of Education