



# The Need for Stringent Quality Guidelines for Local Stingless Bees End-Products

**Assoc. Prof. Dr Mahaneem Mohamed**

Department of Physiology, School of Medical Sciences,  
Universiti Sains Malaysia

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# Outline:

- Types, composition and biological properties
- End-products
- Traditional Medicine/ Natural products
- Quality
- Safety
- Efficacy
- Cosmetics



honey



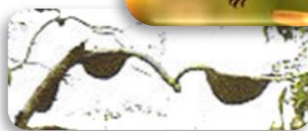
wax



pollen



apilarnil



Bee bread



venom



Royal jelly



propolis

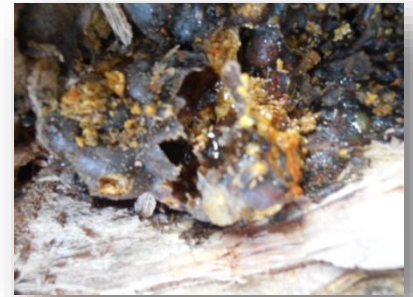
## Honey bees



honey



Stingless bees



Bee bread



propolis

# Composition (honey)

- carbohydrates (fructose, glucose, maltose, sucrose)
- proteins – enzyme glucose oxidase
- minerals – calcium, iron, zinc
- vitamins – A, B, C, E, K
- phenolic compounds (flavonoids & phenolic acids)

# Biological properties (honey)

- antioxidant (protects against free radicals)
- anticancer
- antibacteria
- antiulcer
- antidiabetes

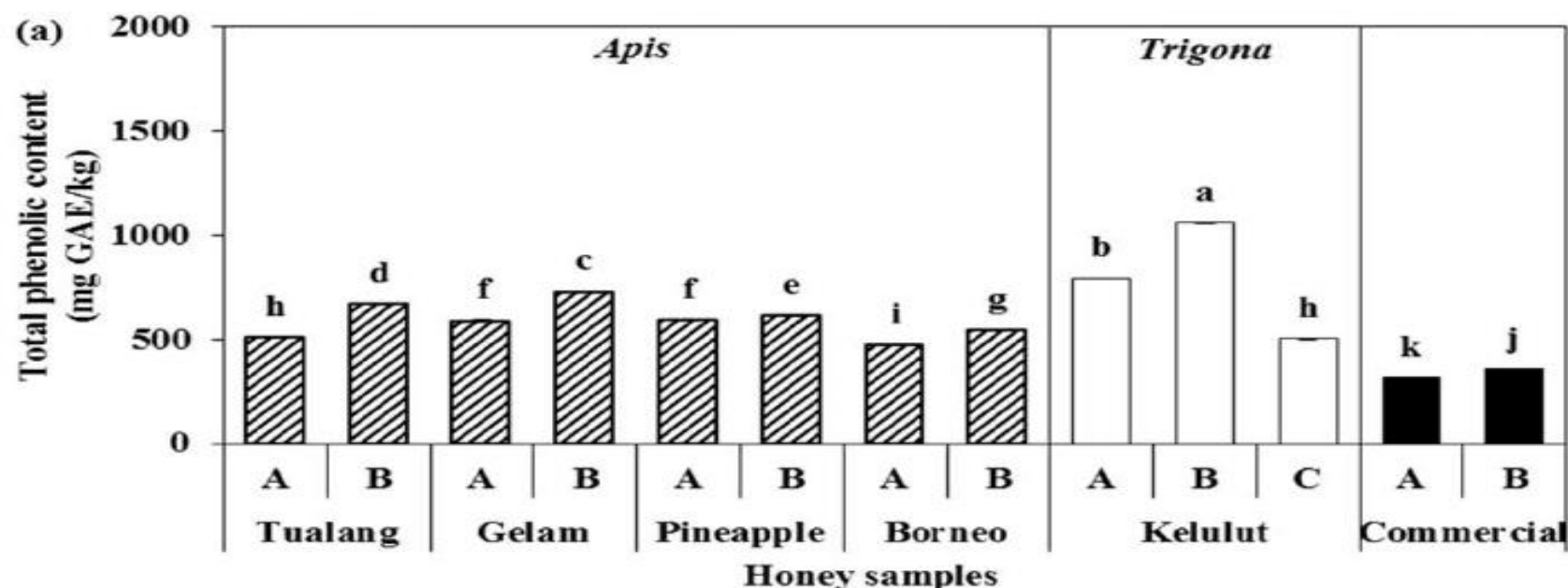
“ST26943”, 2nd International Conference on Agricultural and Food Engineering, CAFEi2014”

## Total Phenolic Contents and Colour Intensity of Malaysian Honey from the *Apis* spp. and *Trigona* spp. Bees

Siok Peng Kek<sup>a</sup>, Nyuk Ling Chin<sup>a,\*</sup>, Yus Aniza Yusof<sup>a</sup>, Sheau Wei Tan<sup>b</sup>, Lee Suan Chua<sup>c</sup>

<sup>a</sup>Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

<sup>b</sup>Laboratory of Vaccines and Immunotherapeutics, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia





## **ANTIOXIDANT PROPERTIES AND INHIBITORY EFFECTS OF TRIGONA HONEY AGAINST *Staphylococcus aureus* PLANKTONIC AND BIOFILM CULTURES**

**\*Wen Jie Ng<sup>1,2</sup>, Yek Jia Chan<sup>1</sup>, Zhi Khoon Lau<sup>1</sup>, Ping Ying Lye<sup>1</sup> and Kah Yaw Ee<sup>1,2</sup>**

<sup>1</sup>Faculty of Science, Universiti Tunku Abdul Rahman, Malaysia; <sup>2</sup>Centre for Biodiversity Research,  
University Tunku Abdul Rahman, Malaysia

**\*Corresponding Author, Received: 9 May 2016, Revised: 22 Aug. 2016, Accepted: 9 Dec. 2016**

**ABSTRACT:** Trigona honey was analyzed for bactericidal and antibiofilm potencies using plate count and spectrophotometry methods, respectively, against different *Staphylococcus aureus* isolates, including ATCC 25923 strain, ATCC 33591 methicillin resistant strain (MRSA), and two clinical isolates from wounds. Besides, the relationship between anti-staphylococcal effects and antioxidant capacity of Trigona honey was discussed. All *S. aureus* isolates were highly susceptible to the antibacterial action of Trigona honey. Lysis of the planktonic bacterial cells was observed using scanning electron microscopy. Despite moderate levels of phenolic content (106.62 mg GAE/kg), DPPH free radical scavenging activity (40.94% RSA), and FRAP value (419.50  $\mu\text{M}$  Fe (II)/100g), Trigona honey exhibited potent inhibitory effect (75-90%) on biofilm formation, especially in 20% (v/v) honey. Additionally, the effects of functional phytochemicals and acidity (pH 2.31) in 20% (v/v) honey were suggested to contribute up to 70% reduction on established biofilm. In short, Trigona honey exhibited high antibacterial and antibiofilm activities, suggesting a potential therapeutic agent in staphylococcal wound infection.

**Keywords:** Stingless bee honey, Antioxidant, Antibacterial, Antibiofilm, Trigona





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Original article

# Pancreatoprotective effects of *Geniotrigona thoracica* stingless bee honey in streptozotocin-nicotinamide-induced male diabetic rats



Muhammad Shakir Abdul Aziz<sup>a</sup>, Nelli Giribabu<sup>b</sup>, Pasupuleti Visweswara Rao<sup>a,c,\*\*</sup>,  
 Naguib Salleh<sup>b,\*</sup>

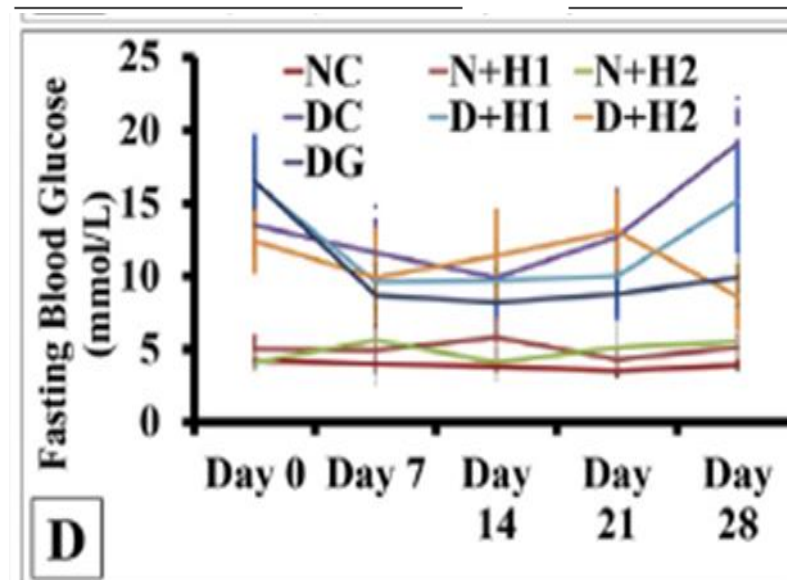
<sup>a</sup> Bio-Industrial Technology Program, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Campus Jeli, 17600 Jeli, Kelantan, Malaysia

<sup>b</sup> Department of Physiology, Faculty of Medicine, University of Malaya, 50603, Lembah Pantai, Kuala Lumpur, Malaysia

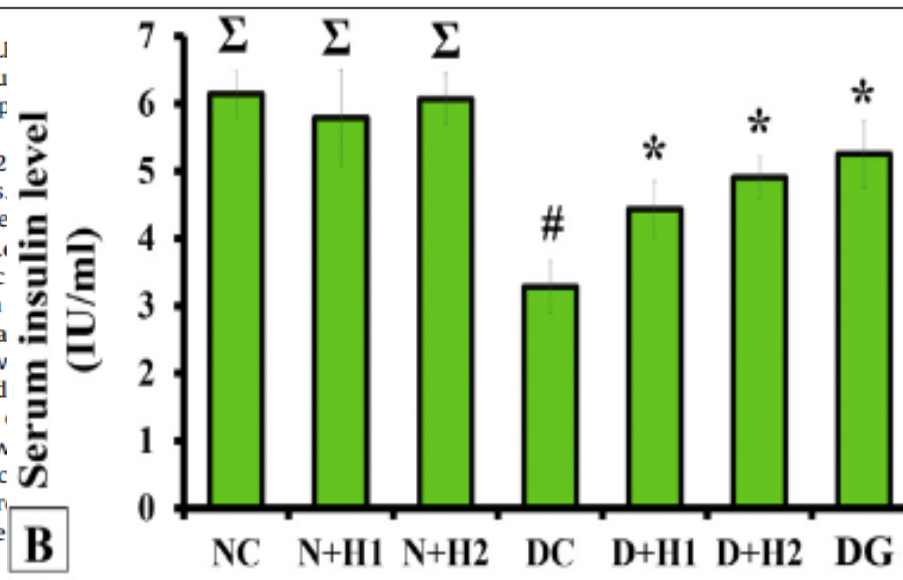
<sup>c</sup> Institute of Food Security and Sustainable Agriculture, Universiti Malaysia Kelantan, 17600, Malaysia

## ARTICLE INFO

## ABSTRACT



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# Composition (propolis)

- phenolic compounds (flavonoids & phenolic acids)
- wax
- minerals – calcium, magnesium, phosphorus

# Biological properties (propolis)

- antioxidant
- antibacteria
- anticancer (breast, oral)
- antidiabetes

Short Communication

**PHYTOCHEMICAL SCREENING AND COMPARISON OF ANTIOXIDANT ACTIVITY OF WATER AND ETHANOL EXTRACT PROPOLIS FROM MALAYSIA**

**UMAR ZAYYANU USMAN, AINUL BAHYAH ABU BAKAR, MAHANEEM MOHAMED\***

Department of Physiology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

Email: mahaneem@usm.my

*Received: 11 Jan 2016 Revised and Accepted: 15 Mar 2016*

**ABSTRACT**

**Table 2: Antioxidants property of Malaysian propolis extracts**

Antioxidant property	WEP	EEP
Radical scavenging activity (%)	70.69±0.49	82.44±0.05 <sup>*</sup>
Total phenolic content (mg gallic acid Eq per g)	119.00±7.00	646.67±30.44 <sup>*</sup>
Total flavonoid content (mg quercetin Eq per g)	87.58±5.20	209.83±1.42 <sup>*</sup>

Data are mean±standard deviation (n=3). WEP: water extract propolis, EEP: ethanol extracts propolis. <sup>\*</sup>P<0.05 compared to WEP (Independent t-test).

## **Phytochemical composition and activity against hyperglycaemia of Malaysian propolis in diabetic rats.**

**Umar Zayyanu Usman, Ainul Bahiyah Abu Bakar, Mahaneem Mohamed\***

Department of Physiology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kelantan, Malaysia.

### **Abstract**

Diabetes mellitus (DM) is a disease associated with hyperglycaemia and loss of body weight. Brazilian propolis is shown to have hypoglycaemic effect in diabetic rats. However, the role of Malaysian propolis on food intake, body weight gain and fasting blood glucose in diabetes has not been reported. We aimed to determine the phytochemical compounds in ethanol extract

Groups	Fasting blood glucose (mg/dl)	
	Before treatment	After treatment
Non-DM	92.17 (3.19)	91.17 (2.56)
DM	435.50 (90.52) <sup>a</sup>	533.17 (70.37) <sup>a</sup>
DM+300EEP	446.17 (31.67) <sup>a</sup>	308.67 (39.38) <sup>a, b</sup>
DM+600EEP	425.00 (81.48) <sup>a</sup>	243.00 (82.00) <sup>a, b</sup>
DM+metformin	519.00 (58.87) <sup>a</sup>	252.50 (63.82) <sup>a, b</sup>

Results are expressed as mean  $\pm$  SD and n= 6 for each group.

## Research Article

# Antioxidant Properties and Cardioprotective Mechanism of Malaysian Propolis in Rats

Romana Ahmed,<sup>1</sup> E. M. Tanvir,<sup>2</sup> Md. Sakib Hossen,<sup>1</sup> Rizwana Afroz,<sup>1</sup> Istiyak Ahmmed,<sup>1</sup> Nur-E-Noushin Rumpa,<sup>1</sup> Sudip Paul,<sup>1</sup> Siew Hua Gan,<sup>3</sup> Siti Amrah Sulaiman,<sup>4</sup> and Md. Ibrahim Khalil<sup>1,3</sup>

<sup>1</sup>Laboratory of Preventive and Integrative Biomedicine, Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh

<sup>2</sup>Veterinary Drug Residue Analysis Division, Institute of Food & Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka 1349, Bangladesh

<sup>3</sup>Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

<sup>4</sup>Department of Pharmacology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian 16150, Kelantan, Malaysia

Parameters	Treatment			
	Control	MP	ISO	MP + ISO
SOD (units/mg of protein)	$1.53 \pm 0.15^a$	$1.96 \pm 0.04^a$	$0.13 \pm 0.02^c$	$0.31 \pm 0.05^b$
GRx (nmol NADPH oxidized/min/mg of protein)	$96.23 \pm 6.70^a$	$94.32 \pm 1.74^a$	$71.07 \pm 4.78^b$	$89.13 \pm 9.28^a$
GPx (nmol NADPH oxidized/min/mg of protein)	$2.95 \pm 0.57^a$	$2.27 \pm 0.52^a$	$0.90 \pm 0.02^b$	$1.98 \pm 0.51^a$
GST (nmol CDNB conjugated/min/mg of protein)	$2.08 \pm 0.20^a$	$2.55 \pm 0.43^a$	$1.29 \pm 0.18^b$	$2.13 \pm 0.63^a$

Data are presented as means  $\pm$  SD,  $n = 8$ .

<sup>a,b,c</sup> Values in the same row that do not share superscript letters (a, b, and c) indicate significant difference at  $p < 0.05$ .

MP: Malaysian Propolis. ISO: isoproterenol.



# Composition (bee bread)

- carbohydrate (fructose, glucose, sucrose)
- proteins (amino acid essentials & non-essentials)
- minerals (calcium, iron, zinc)
- vitamins (A, B, C, D, E, K, Folic acid)
- phenolic compounds (flavonoids & phenolic acids)

# Biological properties (bee bread)

- antioxidant
  - antibacteria
  - anticancer (breast, oral)
  - antidiabetes
- 
- pH 3.5 – 4.2 (lactic acids; fresh pollen pH ~ 7.2)

## ORIGINAL RESEARCH ARTICLE

### Total phenolic content, total flavonoid and antioxidant activity of ethanolic bee pollen extracts from three species of Malaysian stingless bee

Nurdianah Harif Fadzilah<sup>a</sup> , Mohd Fahimee Jaapar<sup>b</sup> , Rosliza Jajuli<sup>b</sup>  and Wan Adnan Wan Omar<sup>a\*</sup> 

<sup>a</sup>Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, Malaysia; <sup>b</sup>Agrobiodiversity and Environment Research Centre, Malaysian Agriculture and Research Institute, Serdang, Malaysia

(Received 13 July 2016; accepted 17 January 2017)

Bee pollen consists of flower pollen mixed with bee digestive enzymes and preserved with some honey and nectar. It contains high antioxidant activity due to the presence of polyphenols and flavonoids. This study was aimed at investigating the chemical profiles of bee pollen extracts from three species of Malaysian stingless bee, *Trigona (Tetrigona) apicalis*, *Trigona (Heterotrigona) itama* and *Trigona (Geniotrigona) thoracica*. Chemical profiles analyzed were total phenolic content

Table 2. Summary of TPC, TFC and antioxidant activity (EC50) in different species.

Species	TPC (mg/g GAE)*	TFC (mg/g QE)*	EC50 (mg/ml)*
<i>T. apicalis</i>	135.93 ± 0.02	25.72 ± 0.17	1.05 ± 0.01
<i>T. itama</i>	33.46 ± 0.02	15.28 ± 0.04	3.24 ± 0.03
<i>T. thoracica</i>	103.62 ± 0.04	31.80 ± 0.13	0.86 ± 0.01
Mean value of the three stingless bee species	91.00 ± 0.03	24.27 ± 0.11	1.72 ± 0.02

\*Results are given as mean and standard deviation of three replicates; *n* = 1.

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## Asian Pacific Journal of Tropical Biomedicine

journal homepage: [www.elsevier.com/locate/apjtb](http://www.elsevier.com/locate/apjtb)Entomological research <http://dx.doi.org/10.1016/j.apjtb.2015.12.011>

## Bee pollen extract of Malaysian stingless bee enhances the effect of cisplatin on breast cancer cell lines



Wan Adnan Wan Omar\*, Nur Asna Azhar, Nurdianah Harif Fadzilah, Nik Nur Syazni Nik Mohamed Kamal

Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, 13200, Penang, Malaysia

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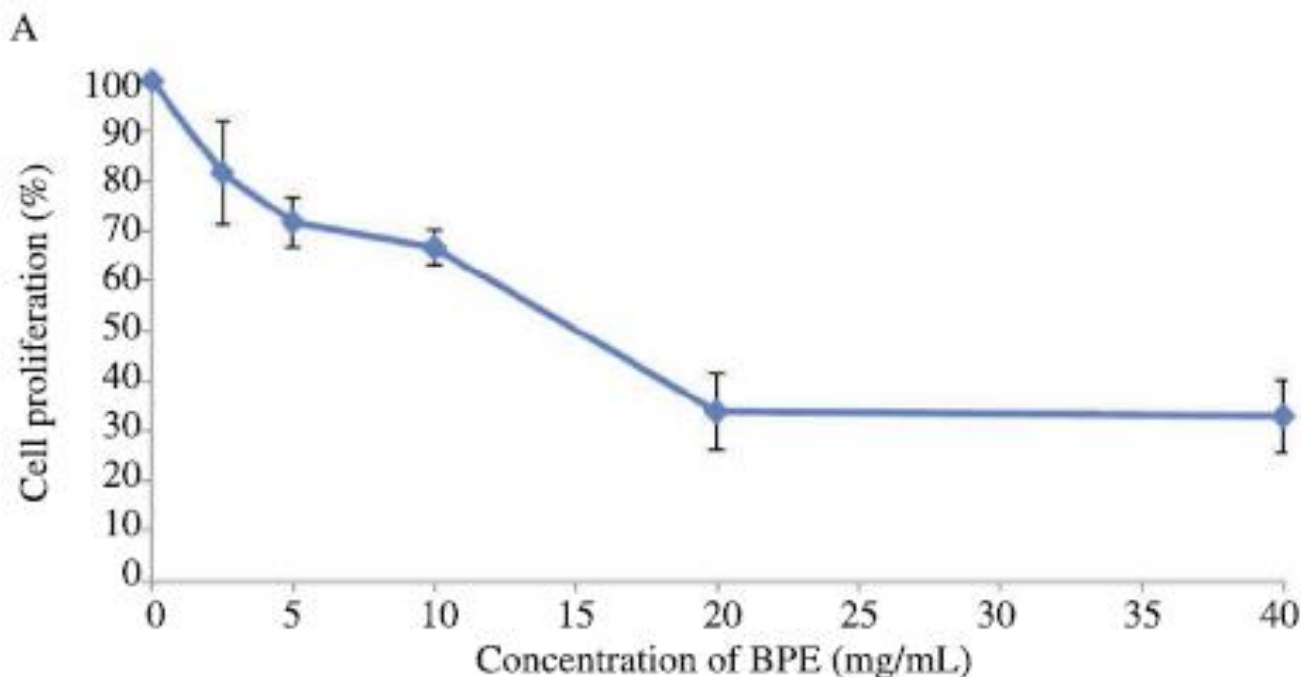
Antipro

Synergi

Malaysi

## ABSTRACT

**Objective:** To evaluate the antioxidant and antiproliferative effect of methanolic bee pollen extract (BPE) of Malaysian stingless bee [*Lepidotrigona terminata* (L. *terminata*)] and its synergistic effect with cisplatin (a chemotherapeutic drug) on MCF-7 cancer cell



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based on

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CHD, Cardiac Fibrosis,  
Hypertension, Ischemia,  
Myocardial infarction

Skin Aging,  
Sunburn, Psoriasis,  
Dermatitis, Melanoma

Chronic Kidney  
disease,  
Renal  
Graft,  
Nephritis

Rheumatoid,  
Osteoarthritis,  
Psoriasis

Asthma, COPD,  
Allergies, ARDS,  
Cancer

Alzheimer, Parkinson  
OCD, ADHD, Autism,  
Migraine, Stroke,  
Trauma, Cancer

Chronic Inflammations,  
Auto-immune disorders,  
Lupus, IBD, MS, Cancer

Restenosis,  
Atherosclerosis,  
Endothelial  
Dysfunction,  
Hypertension

Diabetes,  
Ageing,  
Chronic  
Fatigue

Macular degeneration,  
Retinal degeneration,  
Cataracts

## Free Radical Oxidative Stress

**Heart**

**Skin**

**Kidney**

**Joints**

**Lung**

**Brain**

**Immune  
System**

**Blood  
Vessels**

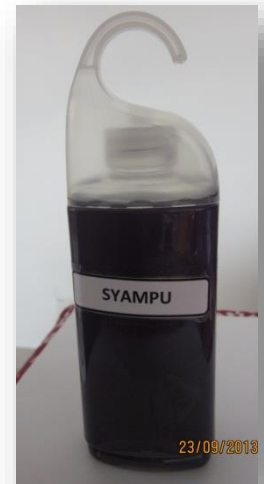
**Multi-  
Organ**

**Eyes**











dehydrator



# Risk

- Allergic reaction
  - itchiness (throat, mouth)
  - numbness especially lips & face
  - swollen face
  - breathlessness
  - acute renal failure (propolis, bee pollen)



## CASE REPORT

### Acute Renal Failure Induced by a Brazilian Variety of Propolis

Yi-Jung Li, MD, Ja-Liang Lin, MD, Chih-Wei Yang, MD, and Chun-Chen Yu, MD

● Propolis is a resinous substance collected by honeybees and used in hive construction and maintenance. Cumulative evidence suggests that propolis may have anti-inflammatory, antibiotic, antioxidant, antihepatotoxic, and antitumor properties. In addition to topical applications, products containing propolis have been used increasingly as dietary supplements. Although reports of allergic reactions are not uncommon, propolis is reputed to be relatively nontoxic. Its systemic toxicity is rarely reported and hence may be underestimated. This is the first report of propolis-induced acute renal failure. A 59-year-old man required hemodialysis for acute renal failure. The patient had cholangiocarcinoma and had ingested propolis for 2 weeks before presentation. Renal function improved after propolis withdrawal, deteriorated again after reexposure, and then returned to a normal level after the second propolis withdrawal. This case indicates that propolis can induce acute renal failure and emphasizes the need for vigilance and care when propolis is used as a medicine or dietary supplement. *Am J Kidney Dis* 46: E125-E129.

© 2005 by the National Kidney Foundation, Inc.

INDEX WORDS: Propolis; acute renal failure.



## A Case Report of Acute Renal Failure Associated With Bee Pollen Contained in Nutritional Supplements

Tsuchida Akiyasu,<sup>1</sup> Bishnuhari Paudyal,<sup>1,2</sup> Pramila Paudyal,<sup>2</sup> Matsumoto Kumiko,<sup>1</sup> Ueki Kazue,<sup>3</sup> Naruse Takuji,<sup>3</sup> Kuroiwa Takashi,<sup>4</sup> Nojima Yoshihisa,<sup>4</sup> and Komai Minoru<sup>1,3</sup>

<sup>1</sup>*Internal Medicine and Kidney Dialysis, Sanshi Group Hikari Clinic, Isesaki, Departments of* <sup>2</sup>*Diagnostic Radiology and Nuclear Medicine, and* <sup>4</sup>*Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, and* <sup>3</sup>*Sanshi Group Toho Hospital, Midori, Japan*

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**Abstract:** We report a case of renal failure associated with the ingestion of bee pollen containing nutritional supplement. A 49-year-old male patient who had been ingesting a nutritional supplement for more than five months had breathing difficulties, anuria, exceptional weight gain (20 kg) due to systemic edema, and loss of appetite. A renal biopsy confirmed interstitial nephritis with the presence of eosinophils, which is suggestive of drug-induced acute renal failure. The nutritional supplement was ceased and hemodialysis begun. The patient's condition improved after

several hemodialysis sessions, which were then stopped. Current information regarding the adverse effects of bee pollen is not very robust, therefore potential damage should be kept in mind before ingesting nutritional supplements in which it is contained. This report serves as an important reminder to the public as well as healthcare providers of the potential of renal failure related to nutritional supplements. **Key Words:** Acute renal failure, Bee pollen, Hemodialysis, Nutritional supplement, Proteinuria.

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Natural/ Traditional Products Required To Be  
Registered as  
Traditional Medicine (not new drug)  
(National Pharmaceutical Regulatory Agency  
(NPRA), Ministry of Health (MOH), Malaysia)

- Traditional medicine in pharmaceutical dosage form with **claims**.
- eg: pill/ tablet, soft gel, capsule, tea bag, powder





# NPRA-Product Codes:

- A: Scheduled Poisons
- X: Non-scheduled Poisons (over the counter products)
- T: Traditional Medicines
- K/KE: Cosmetics
- C: Contract Manufactured
- E: Export Only
- R: Repacked
- S: Second source
- Traditional Medicines (label) **MALXXXXXXXXT**
- Cosmetics (Label) **NOTXXXXXXXXXXK**

- **Traditional medicine** is defined as any product used in the practice of indigenous medicine, in which the drug consists solely of one or more naturally occurring substances of a plant, animal or mineral, or parts thereof, in the **unextracted or crude extract form** and a homeopathic medicines.

# Natural/ Traditional Products : Not Required to be Registered as Traditional Medicine

- Extemporaneous medicine – prepared and **given directly to patient** by traditional practitioner.
- Herbal medicine containing plants produced only through **drying, grinding or blending (raw herbs)**.
- A herbal preparation containing plants normally taken as **food** and consumed as a **drink/beverage** with **no medicinal claim**.
- a preparation used for a **cosmetic purpose** (eg. to whiten the skin) shall be registered as a **cosmetic product**.



# Evaluation of Traditional Medicine/ Natural products

- Quality
- Safety
- Efficacy - based on the claimed benefits and documented use as folk medicines based on philosophy of the respective traditional medicines .

# Quality

- Raw material
- Finished product (excipient? dosage?)
- Extraction – good manufacturing practice certified company, solvent?
- Heavy metal analysis
- Microbial analysis
- Stability testing
- Disintegration test (tablet)

# Safety

- Does not contain banned ingredients
- Warning statements/adverse effects on product label
- Contains substances in allowable limits/dose
- Limits for heavy metals, limits for microbial contamination, no adulterants

Men' health – sildenafil, tadalafil & its analogue

Slimming – fenfluramine

Muscle & joint pain – NSAIDS, steroids

Cough & cold - antihistamine



*Examples of indication allowed (low & medium claims)*  
*Traditionally used.....*

**GENERAL HEALTH MAINTENANCE/ KESIHATAN AM**

- for general health maintenance / for general well being
- for health and strengthening the body

**BLOOD & BODY FLUID / DARAH & CECAIR BADAN**

- for improving blood circulation
- to improve urination
- for improving bowel movement

**BONE, MUSCLE AND JOINT / TULANG, OTOT & SENDI**

- for strengthening muscle and bone
- for relieving muscular ache .
- for relieving waist ache and backache

**SKIN AND EXTERNAL USE**

- for symptomatic relief of pain and itch associated with insect bites

## Examples of indication allowed

Traditionally used.....

### **PAIN & FEVER / SAKIT AM & DEMAM**

- to relieve / alleviate pain
- for relieving headache

### **COUGH & COLD**

- to relief cough and cold
- to relief of nasal congestion

### **DIGESTIVE SYSTEM**

- for relief of stomachache, mild diarrhea
- for relief of flatulence, stomach ache, mild diarrhea and
- loss of appetite

### **WOMEN'S HEALTH / MEN'S HEALTH**

- to relief menstrual pain, headache,
- to relief vaginal discharge
- for energy and men's health/ for vitality



## Non-permissible Indications

1. Penyakit atau kecacatan ginjal / Disease or defects of the kidney
2. Penyakit atau kecacatan jantung / Disease or defects of the heart
3. Kencing manis / Diabetes
4. Epilepsi atau sawan / Epilepsy or fits
5. Kelumpuhan / Paralysis
6. Tibi / Tuberculosis
7. Asma / Asthma
8. Kusta / Leprosy
9. Kanser / Cancer
10. Kepekakan / Deafness

# Non-permissible Indications

- 11.Ketagihan dadah / Drug addiction
- 12.Hernia atau pecah / Hernia or rupture
- 13.Penyakit mata / Disease of the eye
- 14.Hipertensi (Darah Tinggi) / Hypertension
- 15.Sakit otak / Mental disorder
- 16.Kemandulan / Infertility
- 17.Kaku / Frigidity
- 18.Lemah fungsi seks atau impoten / Impairment of sexual function or impotency
- 19.Penyakit venerus / Venereal disease
- 20.Lemah urat saraf atau aduan atau kelemahan lain timbul daripada atau berhubungkait dengan perhubungan seks / Nervous debility or pother complaint of infirmity arising from or relating to sexual intercourse.

e) Example of label approved by the Authority:

This is a traditional medicine

Please consult your pharmacist/  
doctor before taking this product

Jauhkan daripada kanak-kanak  
*Keep out of reach of children*

**Indication:** Traditionally used for  
women's health

**Warning:** Pregnancy and  
breastfeeding: Insufficient reliable  
data

Keep below 30 ° celcius  
Protect from light and moisture

Manufacturing date:  
Expiry date:  
Batch No.:

KAPSUL PQR  
500MG

MALXXXXXXXXT

50 CAPSULE

Hologram

Each Capsule (Vegetable capsule)  
contains :

Folium XX      200mg  
Fructus QY     300mg

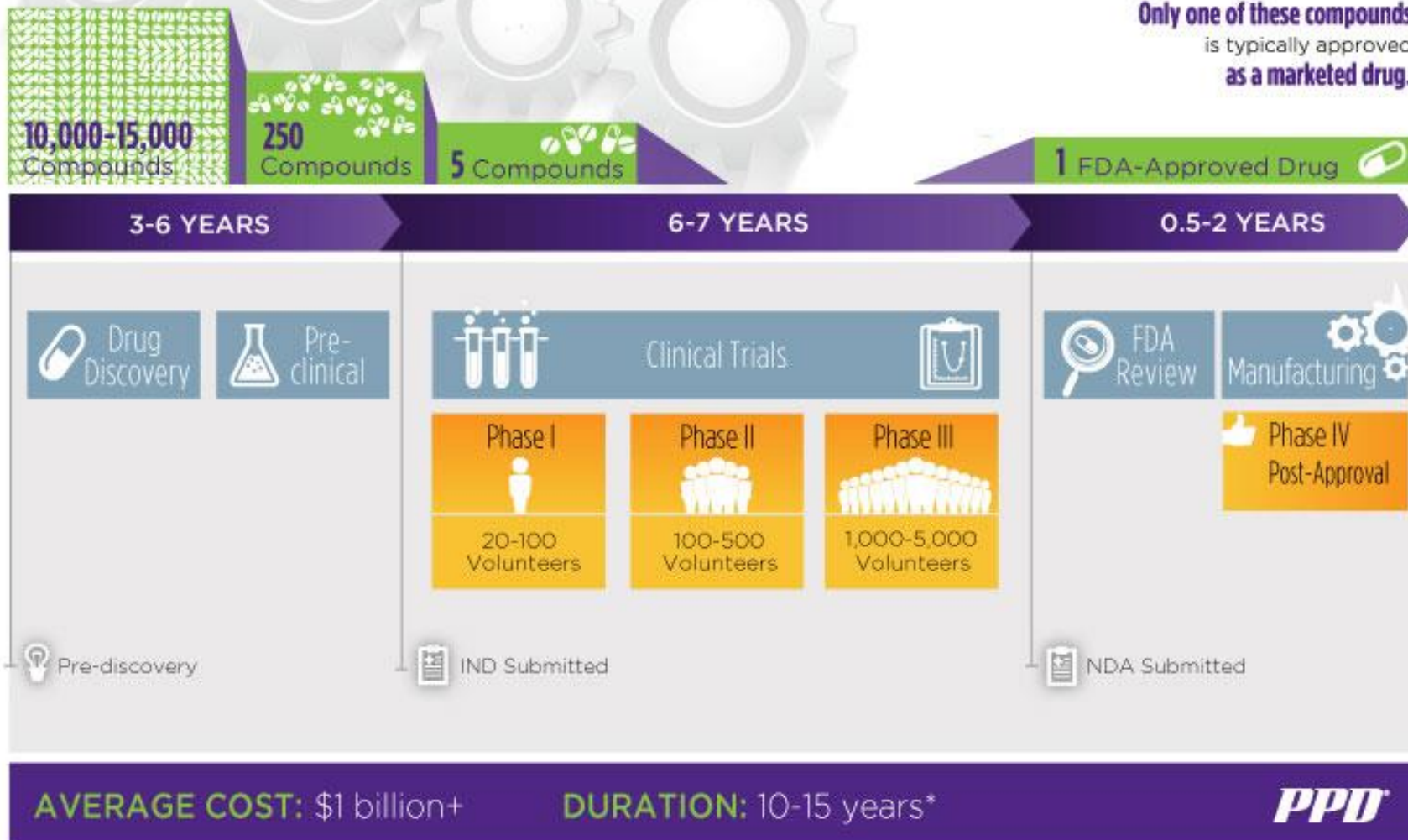
Dosage : 2 capsule taken twice a  
day after food

Marketing authorization holder:  
Syarikat XYZ Sdn Bhd  
18, Jalan Utama  
47000 Sungai Buloh  
Selangor

Manufactured by:  
Syarikat ABC Sdn Bhd  
3, Jalan Universiti  
46730 Petaling Jaya

# DRUG DEVELOPMENT PROCESS

Out of every 10,000-15,000 new compounds identified during discovery, **five are considered safe for testing** in human volunteers. **Only one of these compounds** is typically approved as a marketed drug.



\*Source: ACRO



# Challenges??

- Analysis & standardization of finished products
- Safety and side effects of the formulation (toxicity studies)
- Correct dose & duration of treatment (pre- and clinical studies)
- Mode of action (mechanism of action)
- Drug-natural product interaction

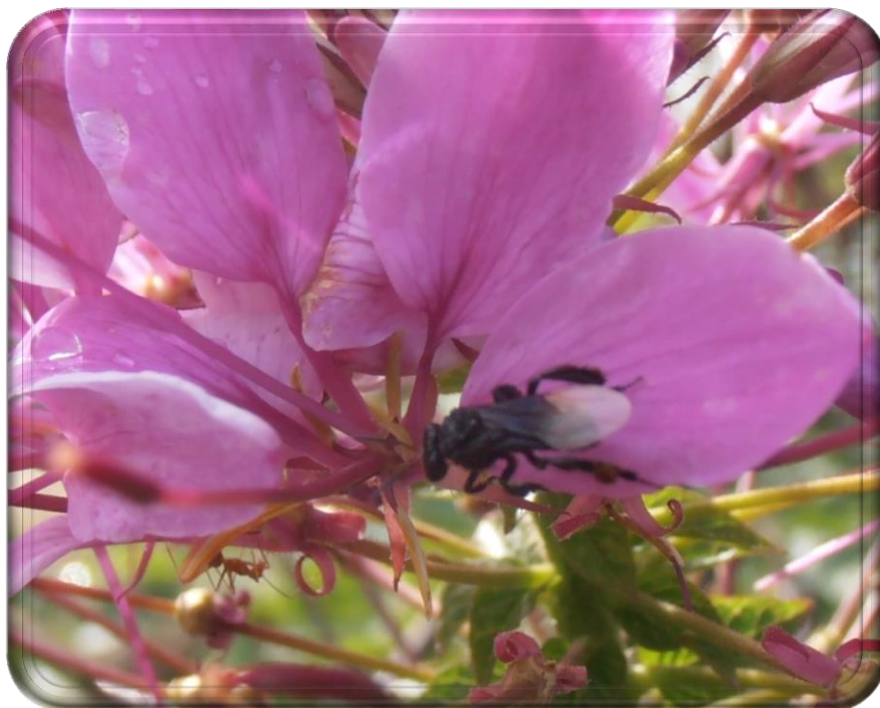
# Cosmetics (Quality)

- good manufacturing practice certified company (contract manufacturing)
- heavy metal analysis
- microbial analysis
- does not contain banned ingredients (mercury, hydroquinone, tretinoin)
- product registration (NOTXXXXXXXXXXK)
- halal?



# Summary

- stingless bee products have medicinal properties and needs further research, budget??
- many end-products can be developed
- challenges – fulfill the requirement
- important to meet the stringent quality guideline
- the sustainability of stingless bee industry
- protect the public from any harmful effects



**Thank you**  
mahaneem@usm.my