PROCEEDINGS

CMU-KU Symposium 2009

The Second International Meeting for Development of International Network for Reduction of Agrochemical Use: Food Safety Technologies in Southeast Asia
September 22-23, 2009
The Imperial Mae Ping Hotel
Chiang Mai, Thailand

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On behalf of Chiang Mai people, I cordially welcome all distinguished participants of the 2nd International Meeting for Development of International Network for Reduction of Agrochemical Use: Food Safety Technologies in Southeast Asia to Chiang Mai, Thailand.

Chiang Mai is often referred to as the “Rose of the North”. Everyone who has visited Chiang Mai admits that it is certainly a land of beauty that was built on the roots of a traditional heritage that dig deep into the soil of time, and with a distinct cultural personality of its own.

Chiang Mai province for years now has been the tourist hub of the north and one of Thailand’s most important tourist destinations. It is considered one of the most scenic provinces in the country due to its mountain ranges, valleys, flora and fauna. Unlike most of Thailand, the climate in the north and especially Chiang Mai is cool, fresh and misty. This year, Chiang Mai is ranking number 5 of the most favorite city in the world to tourists by Leisure magazine.

I wish you all enjoy your stay in Chiang Mai with the best experiences we will share in this meeting at The Imperial Mae Ping Hotel during 22 – 23 September 2009.

Assoc. Prof. Theera Visitpanich
Dean
Faculty of Agriculture
Chiang Mai University
This proceeding book is the fruit of cooperative activities entitled “The 2nd International Meeting for Development of International Network for Reduction of Agrochemical Use: Food Safety Technologies in Southeast Asia” by the researchers from various universities in Japan and Southeast Asia.

East Asian countries are developing remarkably, and increasing demands for safe food and systems to support the quality are eagerly requested for maintaining human health. In the same way, the requirement for healthy food is increasing in Japan and it was become a top priority issue.

Therefore, it is reasonable that scientists from Japan and Southeast Asia cooperate with in their topics and make research groups at this meeting. Since food safety is an interdisciplinary issue, various areas of sciences and technologies, including Agronomy, Food Science, Engineering, Medical Science and Economics should investigate safe food with various techniques and viewpoints.

Chiang Mai University researchers involved in ATRACT (Appropriate Technology for Reduction of Agrochemical in Northern Thailand) JICA project contributed to make the main core of the meeting, and it is an honor that the Faculty of Agriculture, Kagawa University and the Faculty of Bioresources, Mie University collaborated with them.

I would like to express our sincere gratitude to Dr. Theera Visitpanich, the Dean of the Faculty of Agriculture of Chiang Mai University and his staff for energetic hosting of this meeting. I also would like to express thanks to the Japan Society for the Promotion of Science (JSPS) and to the Ministry of Education, Science and Culture, the Government of Japan for financial support of the activity. The exchange activities of young researchers are mainly supported by the Exchange Program for East Asian Young Researchers of the 2009 fiscal year.

Prof. Dr. Shigeyuki Tajima
Dean
Faculty of Agriculture
Kagawa University
INTERNATIONAL FOOD SAFETY NETWORK: FROM ATRACT'S EXPERIENCE TOWARDS REGIONAL COLLABORATIVE DEVELOPMENT

Pittaya Sruamsiri

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KEYNOTE ADDRESS

Japan International Cooperation Agency (JICA) had supported the three-year research and extension project name “Appropriate Technology for Reduction of Agrochemical in Northern Thailand (ATRACT)” to Chiang Mai University in collaboration with Department of Agricultural Extension (DOAE). The project aimed to develop the alternative technologies for reduction of agrochemical use in crucifer, rose, and tangerine farms in northern Thailand. Developed technologies and knowledge were transferred to the farmers through collaboration with DOAE and through the project website. The project was started on 13 November 2004 and last until 12 November 2006.

With the well support from Kagawa University and Mie University as well as Ministry of Agricultural and Cooperatives Thailand (Department of Agricultural Extension), appropriate ways to reduce agrochemicals use in citrus, rose and cabbage production were successfully developed and disseminated to the farmers through Farmers’ Field School.

From the year 2007 onward many research works have been actively undertaken by the CMU group to find alternative biopesticide and biofertilizers from actinomycetes endophyte and beneficial microorganism. Natural insecticide and fungicide from plant extract are also investigated and produced for farm application. The research results showed a very high potential of using those beneficial microorganism and plant extract to ensure food safety, and ready to disseminate to the farmer in Southeast Asian countries.

By the classical sense, “Food Safety” covers “a suitable product which when consumed orally does not cause any health risk”. In a modern society, “Food Safety” is extending to cover “a safer food for a better health”. Functional food for example, becomes then more economic importance worldwide, especially for the new trend society, which dominated by old people due to a longer life-span. Development of food safety in both senses requires a complex knowledge and interdisciplinary technologies, especially when based upon the biodiversity exploring and commercialization. These new challenges require intensive investment and could be well achieved only through the active international collaboration like what was established in the ATRACT achievement.
CMU-KU Symposium 2009

The 2nd International Meeting for Development of International Network for Reduction of Agrochemical Use: Food Safety Technologies in Southeast Asia

22-23 September 2009
Imperial Mae Ping Hotel, Chiang Mai, Thailand

September 22
13.00 Welcome and Opening Address
Dean, Faculty of Agriculture, Kagawa University (KU)

13.10 Keynote address
International food safety network: From ATRACT’s experience towards regional collaborative development
Assoc. Prof. Dr. Pittaya Sruamsiri

SYMPOSIUM:
Development of Food Safety Technologies
13.30 Development of technology for reduction of agrochemicals: Use of rare sugars in agriculture for food safety
Prof. Dr. Kazuya Akimitsu

13.50 Detection of mycotoxins by immunochemical methods
Assoc. Dr. Osamu Kawamura

14.10 Detection of carbendazim-resistant Colletotrichum gloeosporioides causing mango anthracnose disease
Ms. Pornprapa Kongtragoul

14.30 Using multiple funnel trap in combinations with CMU-C1 lure for the surveillance of coffee berry borer
Dr. Yaowaluk Chanbang

14.50 Tea/coffee break

Development of Functional Materials for Food Safety and Health (I)
15.10 Volatiles of Litsea cubeba, possibility as biopesticide and multifunctional additives
Prof. Dr. Hirotoshi Tamura

15.30 Functional components from tropical forest products
Prof. Dr. Takeshi Katayama

15.50 Ellagic acid in longan cv. Daw
Dr. Tanachai Pankasemsuk

16.10 Situation of soybean cultivation in the rubber tree plantation in Cambodia and preliminary study of native soybean root nodule bacteria from this ecosystem
Ms. Monyda Cheach
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Reception
19.00  At The Imperial Mae Ping Hotel

September 23

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   Prof. Dr. Shoichi Gohtani
9.20  Change of gamma-aminobutyric acid (GABA) and glutamic acid decarboxylase (GAD) protein in germinating rice
   Ms. Panatda Jannoey
9.40  Determination of effective plant hormones in liquid biofertilizer
   Dr. Kanokwan Sringarm

Development of Information Technology for Food and Agro-business
10.00 Sensing and monitoring technologies for agricultural applications
   Assoc. Dr. Hideyuki Sawada
10.20 Towards technology for internal detection of mangosteen: A beginning from acoustic signal analysis
   Dr. Nattapong Swangmuang
10.40 Tea/coffee break

Evaluation for Food Safety Technologies and Risk Assessment
11.00 Consumer evaluation for food safety and impact assessment of regional integration
   Assoc. Prof. Dr. Hiroshi Kameyama
11.20 Preliminary investigation of cardiovascular effects and toxicology of Coscinium fenestratum (Gaertn.) Colebr.
   Dr. Natthakarn Chiranthanut
11.40 Conclusion Remarks
11.50 Closing Address
   Dean, Faculty of Agriculture, Chiang Mai University (CMU)

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12.00  At The Imperial Mae Ping Hotel
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Welcome and opening address

Keynote address: International food safety network: From ATRACT's experience towards regional collaborative development
  Pittaya Sruamsiri

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Detection of mycotoxins by immunochemical methods
  Osamu Kawamura

Detection of carbendazim-resistant Colletotrichum gloeosporioides causing mango anthracnose disease
  Pornprapa Kongtragoul, Yoko Miyamoto, Chikako Miyake, Yuriko Izumi, Kazuya Akimitsu and Sarunya Nalumpang

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  Yaowaluk Chanbang, Bantoone Warrit, Chawalit Korsamphan, Warapong Boonma, Prasert Khamon, Nithi Thaisantad, Sombat Srichuwong, Thaworn Supawong and Pishayapa Thongmalai

Volatile of Litsea cubeba, possibility as biopesticide and multi-functional additives
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Iman Hidayat, Yoko Miyamoto, Chikako Miyake, Yuriko Izumi,
Kazuya Akimitsu and Sarunya Nalumpang

Detection \( \beta \)-tubulin gene of carbendazim-resistant *Colletotrichum* spp. causing chilli anthracnose
Wassamon Boonying, Yoko Miyamoto, Chikako Miyake, Yuriko Izumi,
Kazuya Akimitsu and Sarunya Nalumpang
FUNCTIONAL COMPONENTS FROM TROPICAL FOREST PRODUCTS

Takeshi Katayama¹, Syamsul Falah², Nattaya Lourith³,
Mayuree Kanlayavattanakul⁴ and Toshisada Suzuki⁵

¹ Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795 Japan
² Faculty of Mathematics and Natural Sciences, Bogor Agricultural University,
Bogor, 16680 Indonesia
³ School of Cosmetic Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

ABSTRACT

To investigate functional or bioactive components, especially antioxidants, from the bark of mahogany (Swietenia macrophylla King) planted in Indonesia and the seed coats of tamarind (Tamarindus indica L.) in Thailand, which are unused biological resources, the DPPH radical scavenging activity of some extracts from the two materials was assayed. On the mahogany bark, a methanol extract and a hot water extract had higher activity than tocopherol, and the former that had the highest activity is being separated. On the tamarind seed coats, an effective and practical extraction method was investigated. Tamarind seed coat extracts possessed antioxidant activity and especially the activity of the ethyl acetate fractions given by either stepwise extraction or direct maceration was equal to that of vitamin C. Isolation and identification of antioxidants in the two materials are under study. The antioxidants and the antioxidant active extracts are expected for utilization as food additives and supplements as well as cosmetic materials.

Keywords: Antioxidant, Mahogany bark, Tamarind seed coat, Extract

INVESTIGATION OF BIOACTIVE COMPONENTS FROM BARK OF
INDONESIAN FAST GROWING TREES: Utilization of Mahogany (Swietenia macrophylla) Bark Extract as Antioxidant Food Supplements

INTRODUCTION

In some countries, bark has been used as a source of remedies for the treatment of many diseases. The most popular bark used as natural medicines is Cinchona officinalis bark, which has been used as an antimalarial during the past two centuries. Bark has also potential use as an interesting source of strong antioxidants. Pycnogenol extracted from the bark of Pinus maritima has antioxidant activity [1]. Quercetin (a flavone) is a well-known strong
antioxidant and it was isolated from bark of some species such as *Butea frondosa* [2]. In this study, bioactive components from the bark of mahogany planted as a fast growing tree in Indonesia have been investigated. Antioxidant activity from the bark extract was assayed. Falah *et al.* [3] have reported the isolation of swietenemacrophyllanin, catechin, and epicatechin as antioxidants from ethyl acetate (EtOAc) fraction of acetone extract from the bark.

**MATERIALS AND METHODS**

The mahogany bark was stripped from *S. macrophylla* collected from Sumedang, West Java, Indonesia. The chipped bark was ground in a Wiley mill and the resulting meal was screened to give 40-80 mesh bark meal. The bark meal (40-80 mesh) was extracted three times with methanol (MeOH) for 48 h at room temperature, and then with hot water for 1 hr to give a MeOH extract and a hot water extract, respectively. These extracts were separated by chromatographies (silica-gel CC and TLC, and C18-HPLC). The antioxidant activity was assayed for the effect of scavenging stable DPPH free radicals [4]. The samples and tocopherol as a positive reference were dissolved in MeOH to make a 50 ppm solution. Each of the test sample solutions (0, 30, 60, 90, 120, and 150 μL) was added into a mixture (0.9 mL) of 0.4 mM DPPH solution, 20% MeOH aqueous solution, and 0.2 M 2-(N-morpholino)ethanesulfonic acid (MES) buffer solution (1:1:1). The resulting mixtures were shaken on a vortex mixer and stood for 20 min, and then the absorbance of the remaining DPPH in the mixture was determined with a UV-visible spectrophotometer at 517 nm.

**RESULTS AND DISCUSSION**

Figure 1 shows the antioxidant activity of the MeOH extract, a hot water extract, and tocopherol. The MeOH extract had the highest activity among them. Therefore, the MeOH extract was further purified by chromatography. The extract (5g) was chromatographed on a silica-gel column (n-hexane/EtOAc = 80:20 to 0:100 as a stepwise elution) giving 130 fractions. Those fractions were collected to give nine fractions, fr. I (frs. 1-10), fr. II (frs. 11-14), fr. III (frs. 15-20), fr. IV (frs. 21-29), fr. V (frs. 30-34), fr. VI (frs. 35-62), fr. VII (frs. 63-75), fr. VIII (frs. 76-110), and fr. IX (frs. 111-130). Fr. VIII was purified by preparative HPLC [flow rate 2.0 mL min⁻¹, detection: UV 280 nm, eluent: MeOH/H₂O (30:70, v/v)] to afford five fractions (frs. VIII-1 to VIII-5). Fr. IX was rechromatographed on a silica-gel column (MeOH/CH₂Cl₂ = 25:75) to give five fractions (frs. IX-1 to IX-5). Fr. IX-2 was separated by TLC (MeOH/CH₂Cl₂ = 25:75) to afford nine fractions (frs. IX-2-1 to IX-2-9). Fr. IX-3 was separated by TLC (MeOH/CH₂Cl₂ = 25:75) to yield seven fractions (frs. IX-3-1 to IX-3-7). Compounds isolated from those fractions will be identified by NMR, MS, and IR. DPPH scavenging activity of the compounds will be assayed. Antihyperglycemic, anticholesterolemic, and antihyperurisemic activities of the MeOH and hot water extracts will
INTRODUCTION

Tamarind, a fruit tree, is widely distributed in tropical countries including Thailand. This agricultural product or tropical forest product is counted as the economic plant. Due to the diversity of cultivations in Thailand, the fruits bring a variety of tastes and application. Tamarind seeds are the only part that has been abandoned, although its fruits and leaves are consumed as food materials, medication, and cosmetics of which tamarind seed coats possess antioxidant activity [5,6]. However, methods to prepare bioactive compounds are complicated [5]. Therefore, development of simple and practical extraction is performed. The antioxidant activity of each extract was further evaluated. The present beneficial results will be available for application and utilization of the abandon part of tamarind.

MATERIALS AND METHODS

Tamarind seeds obtained by removing the edible part and the pod from the fruit were heated at 140 °C for 45 min, at which the seed coats were separated from the kernels [5]. After cooling down the isolated seed coats were ground into powder, which was extracted as below [5] to give five kinds of extracts in Table 1. Two parts of the powder (each 50 g) were suspended in 70% EtOH, the mixtures was shaken for 30 min by a separatory funnel giving two parts of the extracts. One was concentrated and the other was partitioned between EtOAc and water. The EtOAc layer was concentrated and the aqueous layer was done by azeotropic
distillation with MeOH. The other two parts of the powder (each 100 g) were macerated with 95% EtOH and EtOAc, separately, with shaking at 150 rpm for 24 hr at room temperature.

Antioxidant activity of the extracts was assessed in triplicate as before except for using vitamin C as a positive control [4]. The IC$_{50}$ was shown in means ± S.D. (Figure 2).

RESULTS AND DISCUSSION

Table 1 shows that the 70% EtOH extract was obtained in the higher(est) yield (% w/w) than the extracts given by maceration with 95% EtOH and EtOAc. Although direct maceration with EtOAc is more convenience, the yield given was lower than that of stepwise partition of the 70% EtOH extract with EtOAc.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Weight (g)</th>
<th>% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% EtOH</td>
<td>5.2316</td>
<td>10.46</td>
</tr>
<tr>
<td>EtOAc</td>
<td>0.5135</td>
<td>1.03</td>
</tr>
<tr>
<td>Aq.</td>
<td>5.1132</td>
<td>10.23</td>
</tr>
<tr>
<td>Mac. 95% EtOH</td>
<td>5.3280</td>
<td>5.24</td>
</tr>
<tr>
<td>Mac. EtOAc</td>
<td>0.1589</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Figure 2 DPPH radical scavenging activity of tamarind seed coats: comparison of the extracts with vitamin C.
Figure 2 shows that the EtOAc extract obtained by the partition of the 70% EtOH extract with EtOAc had the highest DPPH radical scavenging activity (IC$_{50}$ of 1.5884 ppm ± 0.200), which was better than that of vitamin C (1.8488 ppm ± 0.470). IC$_{50}$ of the extract by maceration with EtOAc (2.1641 ppm ± 0.290) was almost equal to that of vitamin C. The 70% EtOH extract showed the lowest activity. In contrast, the extract by maceration with 95% EtOH possessed moderate antioxidant activity (IC$_{50}$ = 5.1446 ppm ± 0.440). However, the aqueous fraction had adequate antioxidant activity (IC$_{50}$ = 2.9818 ± 0.268). Therefore, it was suggested that the stepwise extraction could successively isolate the antioxidants and that the direct maceration with EtOAc would be convenient method to obtain antioxidants.

The present antioxidant activity of the seed coats, an unused part of tamarind exhibits the optional choice on sustainable utilization of this agricultural product. In addition, the finding antioxidant property is appropriated for several application not only food additives and supplements but also antiwrinkle and antiaging cosmetics. Therefore, such cosmetic formulation is under development.

ACKNOWLEDGMENTS

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REFERENCES