

PROCEEDING

**International Symposium
on Medicinal Plant and
Traditional Medicine**



**Indonesian Traditional Medicine
for Human Welfare**

Tawangmangu, June 4th - 6th 2014

Jointly organized by



***PROCEEDING
INTERNATIONAL SYMPOSIUM
ON MEDICAL PLANTS AND TRADITIONAL MEDICINE***

INDONESIAN TRADITIONAL MEDICINE FOR HUMAN WELFARE

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Proceeding
INTERNATIONAL SYMPOSIUM ON MEDICINAL PLANTS
AND TRADITIONAL MEDICINE

Indonesian Traditional Medicine for Human Welfare

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MEDICINAL PLANTS AND TRADITIONAL MEDICINE
RESEARCH AND DEVELOPMENT CENTER
In Collaborating With
NATIONAL WORKING GROUP ON MEDICINAL PLANTS
2015

PREFACE

International Symposium on Medicinal Plants and Traditional Medicine was held for two days seminar from June 4-6th 2014 at Tawangmangu, Central Java, Indonesia. The theme of the symposium was Indonesian Traditional Medicine for Human Welfare. The aim of the symposium was to facilitate of brainstorming and information exchange among researcher in medicinal plants and traditional medicine research and development. This symposium held by Medicinal Plants and Traditional Medicine Research and Development Center and collaborating with National Working Group on Indonesian Medicinal Plant. The participants of the symposium were come from the various background both from Indonesia and overseas, namely India, Vietnam, South Korea, and Thailand. The symposium was officially opened by Director General of National Institute of Health Research and Development, Mynistry of Health of Republic Indonesia.

There were five invited speaker from overseas i.e. WHO Representative, Thailand, South Korea, India, Vietnam and three from Indonesia i.e. Chief of National Committee of Jamu Scientification, DR. Trihono, and The Chief of Traditional Medicine Association. Oral presentation were 39 papers and porter presentation were 44 papers respectively. The papers presentation were divided into four main topic such as botany and cultivation technology; medicinal plants phytochemistry; pharmacology; as well as microbiology and biotechnology. This proceeding cover all of the oral and poster presentations.

In addition for two days symposium, there on June 6th, was also held a field trip to visit the research facilities of Medicinal Plants and Traditional Medicine Research and Development Centre such as Medicinal Plant Garden which consist of 850 species of medicinal plants, Aromatic Garden where located at the high altitude of Tlogodlingo area, Jamu Museum, and Post Harvest Laboratory. The participants could gain the lesson learn of all the activities regarding to medicinal plant cultivation, the use of medicinal plant and traditional medicine, and post harvest technology for medicinal plants processing.

In general, this symposium was very successful. The plenary session were broaden the knowledge for all of the participants with newest information dealing with medicinal plants and traditional medicines development. While, the parallel session were provide of information on medicinal plants research finding by reaseracher from various research organization and it was the good forum for exchange of experience among the researcher.

We would like to acknowledge to invited speakers and all the distinguished speakers for their valuable contribution during this conference. Furthermore, we also thank to the steering committee for their advice and support. Finally, we are very grateful and highly appreciate to all participants, paper and poster presenters who participated in the conference as well as cordially contributed by submitting their full manuscripts published in this proceeding. Finally,

we believe that the presence of this proceeding will significantly contribute to the advance scientific research, especially in the field of medicinal plant Traditional medicine.

Tawangmangu, April 2015

Editors

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WELCOME REMARKS
BY
SECRETARY GENERAL
NATIONAL WORKING GROUP OF INDONESIA MEDICINAL PLANTS
At the 46th International Symposium on Indonesia Medicinal Plant,
4 – 6 June 2014, Tawangmangu, Central Java, Indonesia

Your Excellency Minister of Health of the Republic of Indonesia, Ibu Nafsiah Mboi
Your Excellency Minister of State Owned Enterprises Republic of Indonesia, Bapak Dahlan Iskan
Distinguished Governor of Central Java or his Representative
Distinguished WHO Representative to Indonesia
Distinguished Director General National Institute of Health Research and Development, MOH,
Distinguished Director General National Institute of Agriculture Research and Development,
MOA
Distinguished The Regent of Karanganyar District,
Distinguished the Steering Board of the National Taskforce of Indonesia Medicinal Plant
Distinguished Speakers,
Distinguished Participants, Guests,
Ladies and Gentlemen,

Assalamu'alaikumwarahmatullahiwabarakatuh, and best wishes for all of us

The right words at the beginning of the talks is to give thanks and gratitude to God that we all have been given health and maybe present at this symposium in Tawangmangu. This greeting implies that health is embedded in our culture and lifestyle.

Ibu Menteri and Bapak Menteri, Ladies and Gentlemen,
In this great opportunity, I would like to express our thanks to Ibu Minister of Health and Bapak Minister of State Owned Enterprises who has the pleasure to present at this meeting. I wish to thank all participants who have strong enthusiasm to participate in this symposium, especially to guest speakers from South Korea, Vietnam, India and Thailand for their sharing valuable of knowledges and experiences in this symposium. I am proud of welcoming you all in Tawangmangu, the one of the preferred and famous tourist destinations with good scenery and its fresh air.

Distinguished Participants,
May I inform you that Pokjanas TOI has formed since 1990, is one of the non-profit association consisting of representatives from research institutes, universities and relevant ministries, industries and individual experts.

As regular activities, Pokjanas TOI organizes the seminar twice a year, and if implemented in Java then required to be held internationally. The seminar is aimed to exchange the information of conducted research and to apply them to related users for the development of

medicinal plant utilization. Each seminar discussed two medicinal plants and reviewing the research results of the one selected medicinal plant. This symposium will discuss two topics, namely *Litsea cubeba* (krangean) and *Equisetum debile* (horsetail). Krangean fruit is a medicinal plant for aphrodisiac and common cold, that grows native exotic and endemic to the slopes of Mount Lawu, while the Horse tail is used to reduce joint pain inflammation and as a source of calcium for people with osteoarthritis and calcium deficiency.

Distinguished Guest and Participants,

Other than two above main medicinal plant subjects, other research themes will also be granted. The special topic that will be discussed within this 3 days seminar is "Indonesia Traditional Medicine for Human Welfare".

As we know that beside for health program, medicinal plants have multifunctions and multi player effects such as green-environment, green-economy, health-tourism, agro-tourism and also has been proven to increase the household income as well as to strengthen the people-economy. More over, we do hope that the research not only stop on the scientific publication but also continue to the downstream of end product that has the economical value or New Chemical Entity (NCE), particularly to encourage national self-reliance of medicine raw materials. The sequences of medicinal plant and traditional medicine research and development activities involving research, development, design, prototype, trial, stimulating the growth of herbal industry, and service support should be able to create competitive products, acceptable by market both domestically and internationally, and accessible for health service.

But on the other side, it is still a very long way for the use of herbal medicine nationally. Let's look at the reality. Number of experts are quite enough, medicinal plant resources are very abundant but we are still fighting to get raw materials, we are also able to master the technology, then, what makes us very slow to move forward? The spirit and the willingness? Budget? Evidence of clinical data? Lack of medicinal plant farmers?

Okay, let's remove the constraints, but how?

I still remember Ibu Nafsiah Mboi said: "People will be healthy and prosperous if all stakeholders work together"; and Bapak Dahlan Iskan said: "Indonesia would be great if all the potential incorporated in the full coordination". These are the wonderful songs which always sung by the Indonesian best singers.

Ibu Menteri, Bapak Menteri and All the Participants,

We confess that the data on the safety and efficacy of Jamu (herbal medicine) are still very limited. Standardized medicinal plants are also few in number. Similarly, data of research institutions' profile, publications, "research-gate", and all the mutually informations regarding the development Jamu of are scattered and each still need to be completed.

Considering these conditions, the Ministry of Health, Republic of Indonesia and WHO Representative to Indonesia creating new-model of Jamu database called *JamuNet* that is accessible to the world. To that end, we plead to the Minister of Health respectively, after the opening of this symposium officially, may be simultaneously launched the *JamuNet*.

On the other hand, the support of BUMN will guaranteed the acceleration and the utilization of Jamu in order to maintain the health of people, reduce the costs for treatment, Jamu dosage forms, modernization of Jamu and strengthening the people's economy
Ladies and Gentlemen,

On behalf of the POKJANAS TOI, I would like to express our appreciation to the contributions of many individuals and Institutions as well as sponsors. I also greatly appreciate for the tireless effort of the committee of MPTMRDC in organizing this International Symposium Finally, I do hope this seminar may contribute to the development of the utilization of Medicinal Plants and Jamu in Indonesia.

Tawangmangu 4th June, 2014
National Working Group of Indonesia Medicinal Plants,

Indah Yuning Prapti, S.KM., M.Kes.
Secretary General

THE EFFECT OF CROCATIN AND DEACETYL CROCATIN ISOLATED FROM RED BETEL (*Piper crocatum*, RUIZ & PAV.) LEAVE ON MICE ANTIBODY TITER

Yustina¹, Sri Hartini^{1*}, Subagus Wahyuono², Sitarina Widyaningrum³ and Agustinus Yuswanto²

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Abstracts

The aim of this research was to investigate antibody titer effect in mice treated with crocacin and deacetyl crocacin isolated from red betel (*Piper crocatum* Ruiz & pav.). The Balb/c mice immune response were induced with *Listeria monocytogenes*. Antibody titer effect was tested using mouse IgG elisa kit. The effect of both crocacin and deacetyl crocacin IgG titers, at the dose of 2,5; 5; and 10 mg/kg BW, occurred at 10th days after *L. monocytogenes* infection. Both compound showed no significant difference compared to the control group on day 21th after *L. monocytogenes* infection.

Keywords : *Piper crocatum* Ruiz & Pav., crocacin and deacetyl crocacin, IgG titer

INTRODUCTION

The activity of the compounds in the extract of red betel leaf (*Piper crocatum* Ruiz & Pav) was reported (Wicaksono *et al.*, 2009; Rachmawaty *et al.*, 2013). Its immunomodulatory activity was also reported (Hartini *et al.*, 2013a). In general, plants that have immunomodulatory activity has a stimulating activity of specific and non-specific immunity (Wagner & Proskh, 1985). Some of these plants stimulate the humoral and cellular immunity, while others simply activate the cellular components of the immune system, such as phagocytosis function without effect on humoral and cellular immunity (Bafna & Misrha, 2004). The two compound isolated from red betel leaf (crocacin and deacetyl crocacin) activate the phagocytic function (Hartini, *et al.*, 2013b). This research aim to know the effect of crocacin and deacetyl crocacin on humoral immunity.

MATERIALS AND METHODS

Preparation of methanol extract of red betel leaves was done by maceration. The extract was further fractionated by the method of Vacuum Liquid Chromatography, successively using n-hexane, chloroform, ethyl acetate, and methanol. Crocacin and deacetyl crocacin are in the 3rd and 4th of 5 methanolic extracts fractions. Isolation of the two compounds was conducted by preparative Thin Layer Chromatography.

Male Balb/c mice 8 weeks old weighing about 20-25 g and *Listeria monocytogenes* were used for the experiments. All procedures were approved by The Ethical Clearance Commission for pre-clinically research of Laboratorium Penelitian dan Pengujian Terpadu Gadjah Mada

University, Yogyakarta, Indonesia. In the preliminary study, Balb/c mice were divided into treatment group and control group. The treatment group, received 10 mg/kg BW deacetyl crocadin while the control group received 0.7 ml of 1% sodium carboxy methyl cellulose as solvent control, per oral for 14 days. On 15th day (=day 0), 0.2 ml *L. monocytogenes* containing 5×10^3 cfu/ml are injected intraperitoneally to all mice. On day 0, day 3, day 10 and the twenty-one days after *L. monocytogenes* infection, 0.5 ml of blood was taken from the infra-orbital plexus of mice.

In the main study, Balb/c mice were divided into nine groups. Group A, received 2.5 mg/kg BW crocadin, Group B, received 5 mg/kg BW crocadin, Group C, received 10 mg/kg BW crocadin, Group D, received 2.5 mg/kg BW deacetyl crocadin, Group E, received 5 mg/kg BW deacetyl crocadin, Group F, received 10 mg/kg BW deacetyl crocadin, per oral for 14 days. Group G, didn't received drugs, as normal control, Group H, received 0.7 ml of 1% sodium carboxy methyl cellulose per oral as solvent control, and Group I, received 100 mg/kgBW product-X® (contain echinacea extract) per oral as positive control. On 15th day (= day 0) and 25th day 0.2 ml *L. monocytogenes* containing 5×10^3 cfu/ml are injected intraperitoneally to all mice. On day 0, day 10 and the twenty-one days after *L. monocytogenes* infection, 0.5 ml of blood was taken from the infra-orbital plexus of mice.

The humoral immune response determined by measuring the titer of immunoglobulin G (IgG). Measurement of IgG titers using mouse IgG elisa kit. The data were analyzed by one-way ANOVA followed by Tukey test.

RESULT AND DISCUSSION

The compounds isolated from red betel are neolignan. The scientific name of red betel is *Piper crocatum* Ruiz & Pav., so that isolate 1 was named crocadin while isolate 2 was named deacetyl crocadin. The existence of an acetyl group (OCH₃) at C₁ to distinguish crocadin of deacetyl crocadin having hydroxyl groups (OH). The chemical structure differences crocadin and deacetyl crocadin are shown in Figure 1. Crocadin is 2-allyl-4-(1'-hydroxy-1'-(3", 4", 5"-trimethoxyphenyl) propan-2'-yl) -3,5-dimethoxycyclohexa-3, 5-dienone and deacetyl crocadin is 2-allyl-4-(1'-acetyl-1'-(3", 4", 5"-trimethoxyphenyl) propan-2'-yl) -3,5-dimethoxycyclohexa- 3,5-dienone (Kustiawan, 2012). Aside from the relatively high rendement, size crocadin and deacetyl crocadin spotting on TLC chromatogram is relatively large and the color intensity of damping patches on UV detection at 254 nm is very strong. Processes, equipment, and means of detection crocadin and deacetyl crocadin fairly simple, allowing the two compounds used as chemical markers for leaves of *Piper crocatum*. Crocadin and deacetyl crocadin can be used as a marker compound, which is a therapeutic components for *Piper crocatum*.

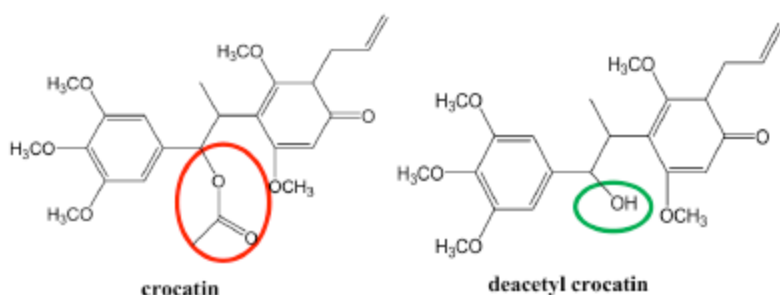


Figure 1. The chemical structure differences between crocetin and deacetyl crocetin.

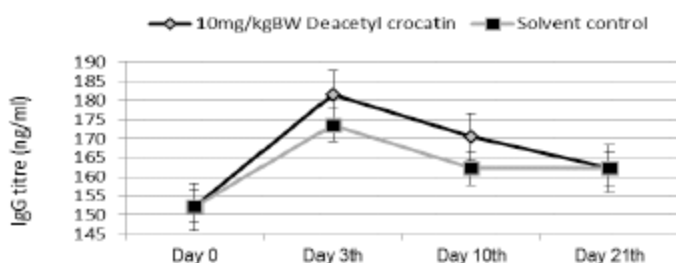


Figure 2. IgG titer levels after the mice were infected by *L. monocytogenes*.

Figure 1 shows the result of preliminary study. In this study, IgG titers of mice treated with 10 mg/kgBW deacetyl crocetin showed increase on day 3th, then decreased on day 10th and it was as same as the control group on day 21th. Although any differences IgG titers on day 3th and 10th, but statistically analysis showed no significant difference between treatment group and control group. It indicates that treatment with 10 mg/kgBW deacetyl crocetin have no IgG titers differences compare to control group. Probably due to on the day-10, it need to boost the mice immune responses, so that in the main study we use twice *L. monocytogenes* infection. In the preliminary study the dose of 10 mg/kgBW deacetyl crocetin showed increasing IgG titer, in order to know the potential level of the compound, we use 2 lower doses in the main study. The main study tested 3 range doses of crocetin and deacetyl crocetin ie : 2,5; 5; and 10 mg/kgBW. The result of main study can see on Figure 3.

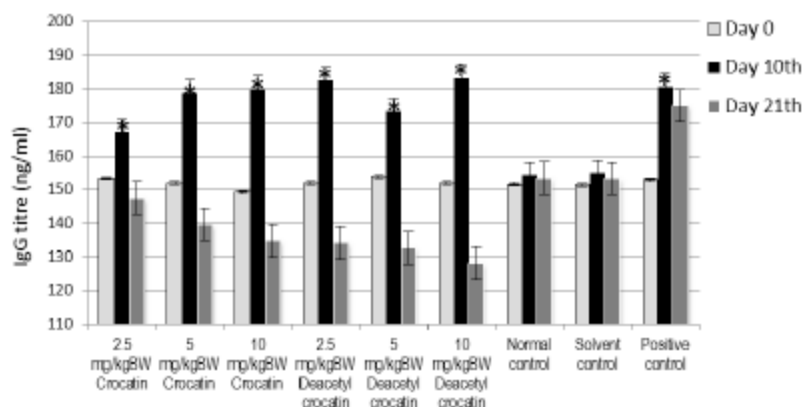


Figure 3. The effect of crocatin and deacetyl crocatin against IgG titers in mice after twice infection with *L. monocytogenes*. Values are mean \pm SD of 3 replicate, * denotes significant difference ($P < 0.05$) to the normal control and the solvent control.

The normal control and solvent control showed the same level of IgG titers, the solvent did not give unexpected effect, 1% sodium carboxy methyl cellulose is an appropriate solvent for this study. In the day 0 (before infection with *L. monocytogenes*) there are no differences effect on all of groups. There are no differences IgG titers of mice before *L. monocytogenes* infection, it indicates that treatment with crocatin, deacetyl crocatin (at dose of 2.5; 5; 10 mg/kgBW) and product-X* (contain echinacea extract, at dose of 100 mg/kgBW) per oral for 14 days, didn't effect on IgG titers. At day 10 after infection of *L. monocytogenes*, the treatment group showed significantly different IgG titers, but on day 21th IgG titers decline, in contrast to the control group but the difference was not significant. Possibly because of the amount of microbial increased on day 10, and then on day 21 had a decline. According Unanue (1997), curve number of *L. monocytogenes* were alive after 0-14 days in mice infected with *L. monocytogenes* showed a slight decrease and then rose on the third day until day 10 reached a peak, and then decreased on day 14 reached zero.

Echinacea is reported to have no effect on the stimulation of IgG immune response, one week following the secondary sheep RBC's subcutaneously infection (Dennis, 1999). Our study using *L. monocytogenes*, an intracellular bacteria, for antigen. Although the test result showed similarity on day 21 after infection antigen, but on day 10. These differences may lead to differences in test result. *L. monocytogenes* induce the cellular immune responses, maybe the humoral immune response wasn't stimulated therefore no effect on the IgG titer.

There are no differences effect of crocatin and deacetyl crocatin on the IgG titers of mice infected with *L. monocytogenes*. Both of the neolignans didn't show significantly effect on the mice IgG titer on the 21th day after *L. monocytogenes* infection, compare to control group.

Probably due to *L. monocytogenes* is an intracellular microorganisms, so that it effect on the cellular immune response but humoral response. As it has reported, the differences of both neolignan are crocatin not cause toxic effects on the kidneys and liver either, but deacetyl crocatin that have OH at C₁ cause liver damage even though safe for the kidneys (Hartini et al, 2013b).

CONCLUSION

There are no differences effect of crocatin and deacetyl crocatin on the IgG titers of mice infected with *L. monocytogenes*. Both of the neolignans didn't show significantly effect on the mice IgG titer, compare to control group.

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