PROCEEDING

International Symposium on Medicinal Plant and Traditional Medicine

Indonesian Traditional Medicine for Human Welfare

Tawangmangu, June 4th - 6th 2014

Jointly organized by

BHAKTI HUSADA

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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 facilitae 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 Commitee 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 MedicineResearch and Development Centre such as Medicinal Plant Garden which consist of 850 species of medicinal plants, Aromatic Garden where located at the high altitute od 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 INDONESIAMEDICINAL 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 decifiency.

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 Wellfare”.

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 scaterred 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

FOSTERING INTEGRATION OF THAI TRADITIONAL MEDICINE INTO HEALTH SERVICE

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Director, Office of International Cooperation Department for Development of Thai Traditional & Alternative Medicine Ministry of Public Health, Thailand

1. The revival of Thai traditional medicine in the health care system

Unlike traditional Chinese medicine of China and Ayurvedic medicine of India, the continuous use of Thai traditional medicine (TTM) for the health care of Thai people was disrupted around early 20th century after the first modern medicine hospital was established in Thailand and the teaching of TTM in medical school was abandoned. The revival of TTM began around 1978 after the proclamation of the Declaration of Alma-Ata when the World Health Organization (WHO) urged member countries to develop and use primary health care (PHC) program as a means to achieve the goal of "Health for All by the Year 2000". Traditional medicine and practitioners were also considered as a part of PHC. Thailand's Ministry of Public Health responded to WHO‟s call by including such a policy to promote the use of selected medicinal plants in PHC since the time of the 4th Health Development Plan (1977-1981). Government policy on the promotion of the use and research and development of medicinal plants and later the use of TTM in the country‟s healthcare system has continued until today as stated in the 5th – 11th (current) National Health Development Plan (2012-2016).

2. The establishment of responsible office and universal health coverage

In 1989, the Ministry of Public Health established the "*Collaborating Center for the Development of Thai Traditional Medicine and Pharmacy*" which was later upgraded to the division level in 1993 as the "*Institute of Thai Traditional Medicine*" (ITTM). Through the years, the institute has organized several activities to develop TTM in various aspects. In October 2002 as a result of the Bureaucratic Reform Act, the "*Department for Development of Thai Traditional and Alternative Medicine*" (DTAM) was established as a new department under the Ministry responsible for the provision of TTM and alternative medicine as other means of health care in the health service system for Thai people.

Also in 2002, the *National Health Security Act* was promulgated in November. Consequently, the *Universal Health Coverage Scheme*, with the largest number of beneficiaries (48.6 million or 75.5% of population), was officially and institutionally established with the National Health Security Office (NHSO) serving as the state (autonomous) agency under the authority of the National Health Security Board (NHSB). TTM and selected alternative medicine services have been covered since the beginning of UHC scheme in Thailand as it was stated in the Article 3 of the Act that "**health services**" included also „Thai traditional and alternative medicine services pursuant to the Practice of the Art of Healing Act‟.

The types of TTM & alternative medicine services covered by UHC are :

- The examination and diagnosis with TTM and applied TTM
- The treatment and rehabilitation with :
    - Herbal medicinal products in the National List of Essential Medicines (NLEM)
    - Nuad Thai (Thai traditional massage) for treatment and rehabilitation
    - Herbal steam bath for therapeutic purpose
    - Hot herbal compress for therapeutic purpose
    - Hot salt pot compress for post-partum care (latest modality added in 2012)
    - Acupuncture of traditional Chinese medicine

In addition to UHC scheme, TTM and alternative medicine services are also covered by the remaining two health security systems; namely, *Civil servant medical benefits scheme* (including also their parents and children < 20 years of age, total about 5 million people) and *Social security scheme* of the Social Security Office for employees of private business (about 10 million people, depending on the country economic situation). Similarly, the above-mentioned treatment modalities, except hot salt pot compress for post-partum care, are also covered by the latter two schemes as well.

Patients under UHC scheme do not have to pay for the above-mentioned services. Health care personnel who can order the above-mentioned TTM treatment modalities for patients are modern medicine doctors and TTM doctors or applied TTM doctors who work in different levels of public hospitals, including „tambon (sub-district) health promotion hospitals" (formerly health centers). For acupuncture, modern medicine doctors who received formal training in acupuncture are the only group of health care personnel in the health care system who can give acupuncture for patients.

To promote the use of TTM and self-reliance on health care of the country, in 2007 the NHSO established the "**Fund for the Development of Thai Traditional Medicine System**" providing additional „**on-top' funding** as an incentive for public health service facilities that provide TTM services in order to stimulate provision of TTM services for out-patients, especially Thai traditional medicines and herbal medicines, Thai traditional massage for therapeutic and rehabilitative purposes as well as post-partum care. The fund is under the supervision, administration and guidance of the Subcommittee on Thai Traditional and Alternative Medicine of the Health Security System. The on-top funding has gradually increased from 0.50 baht/capita in 2007 to 8.19 Baht/capita in 2014 as shown in Table 1.

Table 1. On-top budget allocated from NHSO to public health service facilities to promote the provision of TTM services

Fiscal year	'On-top' fiscal budget allocate from NHSO fund	
	Per capita (baht)	Total budget (million baht)
2007	0.50	28.20
2008	1.00	46.46
2009	1.00	47.02
2010	2.00	94.48
2011	6.00	287.00
2012	7.20	348.00
2013	7.20	348.80
2014	8.19	400.10
2015 (tentative)	9.65	470.69

3. Situation of Thai traditional medicine service in the health care system

In 2012, there are 10,692 public health service facilities providing TTM services. Of these are 100% of regional or general hospitals (94) and community hospitals (740), and more than 80% of tambon (sub-district) health promotion hospitals (equivalent to health center/stations, total number are about 10,000).

During the last fiscal year (2013 from October 2012-September 2013), the KPI of DTAM which was the average percentage of out-patients receiving standard Thai traditional medicine and alternative medicine services in public health service facilities in 12 health service regions was set at 14%. The average percentage of 14.05% was finally achieved with the minimum of 9.4% and the maximum of 20.34% in region 1 and 9, respectively. For fiscal year 2014, KPI is set at 16%.

Regarding the value of herbal medicines used in all public hospitals, it is still a small fraction of all medicines used or less than 2% of all medicines used or about 300 million Baht (about 10 million US$). In community hospitals and tambon health promotion hospitals, the value is higher or about 4.5%. This is because herbal medicines are much cheaper than modern medicines and the use of herbal medicines in the health service system is still much less than that of modern medicines.

4. What DTAM and its network have done to promote and strengthen the integration of TTM and alternative medicine in the health care system

As the national authority on Thai traditional medicine and alternative medicine, the aim of DTAM is "for Thai people to become healthy and self-reliant on health care through appropriate use of TTM and alternative medicine". In order to reach that goal, DTAM and its network have carried out many activities to promote the integration and the use of TTM & alternative medicine in the health care system focusing on the 4 main areas, namely, the 3Ps : **practice** (**service), practitioners** and **products**, and

knowledge generation and management.

Previous work and current activities of DTAM and its network are summarized as follows :

4.1. Activities to promote, improve and facilitate TTM practice and services

4.1.1. Established *collaborative networks* with public hospitals, schools that teach TTM in various universities, related organizations; e.g. National Health Security Office (NHSO), National Health Commission Office of Thailand, Thai Health Promotion Foundation, Ministry of Agriculture and Cooperatives, Ministry of Natural Resources and Environment, Ministry of Commerce, Ministry of Industry, etc. to facilitate collaborative activities,

4.1.2. Collaborated with the Office of the Civil Service Commission to *establish government official positions for TTM* doctors as a new position of health care personnel in the civil service system,

4.1.3. Developed guidelines *and tools to standardize and facilitate TTM services* in public health service facilities, i.e.

- 2003 - Standard of Thai traditional medicine service in public health service facilities
- 2007 - Clinical Practice Guideline of Thai Traditional Medicine (CPG-TM)
- 2008 - The 10th Revision of the International Classification of Disease on Thai traditional medicine (ICD-10-TM)
- 2010 - List and code of Thai traditional medicines
- 2012 - Standard price of Thai traditional medicine and Diagnosis-Related Groups of Thai traditional medicine (DRG-TM) for in-patient care
- 2014 - Unit cost of TTM services
- Standards of Thai Traditional Medicine Hospitals under the Ministry of Public Health, Ministry of Education, and private administration

4.1.4. Supported the provision of TTM services in Tambon (sub-district) Health Promotion Hospitals (collaboration between DTAM, NHSO, Office of the Permanent Secretary),

4.1.5. Developed *pilot TTM Hospitals* and set up OPD and IPD systems of the hospitals. As of 2014, there are 14 pilot TTM hospitals, 12 of which are under Ministry of Public Health and 4 are under Ministry of Education. In 2015, 8 more pilot TTM hospitals are planned to be established in the remaining health service regions that have not yet had TTM hospitals. Lessons learned from the pilot TTM hospitals will pave the way for the establishment of more TTM hospitals nationwide in the future,

4.1.6. Selected more herbal medicinal products into the National List of Essential Medicines (NLEM) to promote the use of traditional/herbal medicines in public health settings. As of May 2014, there are 74 items of licensed herbal

medicinal products and herbal hospital formularies, 50 of which are traditional medicine preparations while 24 are single herbal medicines.

Activities initiated in 2014 are,

4.1.7. Develop *Service Plan of TTM* services in each level of care (primary, secondary and tertiary care) and level of hospitals (tambon (sub-district) health promotion hospitals, community hospitals, general/regional hospitals, and TTM hospitals)

4.1.8. Establish *TTM OPD* in parallel with modern medicine OPD in public hospitals, TTM OPD should open at least twice a week with at least one TTM doctor to provide service (treatment of common diseases and chronic diseases), and not less than 30 items of traditional/ herbal medicines to prescribe. It is expected that at the end of fiscal year 2014, 50% of public hospitals (community and general/regional levels) will establish parallel TTM OPD.

4.1.9. Develop "*DTAM Standards of Nuad Thai*" as a tool to assess and certify NThai facilities according to the standards established,

4.1.10. DTAM & networks join "*the* Princess *Mother's Medical Volunteer Foundation*" to provide TTM services to Thai people in remote rural areas

4.2. Activities to strengthen the knowledge and potential of TTM practitioners & personnel

Undergraduate training

4.2.1. Collaborate with universities offering Bachelor‟s Degree of Thai traditional Medicine or Applied Thai traditional medicine to produce more graduates in TTM to work in the health service system. As of 2014, there are 24 accredited universities that offer Bachelor‟s Degree course, of which 16 are in TTM and 8 are in Applied TTM.

4.2.2. Collaborate with university consortium and NHSO to establish standard of training centers for TTM professional experience and set up certified training centers,

4.2.3. Collaborate with Faculty of Medicine Siriraj Hospital, Mahidol University to develop training course for TTM doctors who work in public or university hospitals that are training centers so that they will become qualified trainers in clinical practice of TTM for TTM students,

4.2.4. Train junior and senior TTM students to be researchers to conduct in-depth interview of folk healers in their communities and conduct case studies on their practices in order to transfer and compile indigenous medicine knowledge of the country.

Post-graduate training and continuing education

4.2.5. Give teleconference continuing education training on special topics of TTM practices for TTM doctors working in public hospitals all over the country,

4.2.6. As of 2014, 5 universities offer graduate degree (M.S. & Ph.D.) courses in TTM or applied TTM,

4.2.7. Provide scholarship and request for government-sponsored scholarship for DTAM officials to study for M.S. and Ph.D. degrees in related fields in Thailand and abroad.

Training for other healthcare personnel

4.2.8. Develop a continuing education course for TTM assistants to be qualified to take a licensing exam to become TTM doctors,

4.2.9. Provide TTM training for medical doctors, physiotherapists, TTM doctors, and nurses,

4.2.10. Request Medical Council to allocate more lecture hours on TTM for medical students to promote better understanding of TTM for future medical doctors so that they can integrate TTM with their modern medicine practice,

4.2.11. Develop curriculum for medical doctors for residency training in preventive medicine in Thai traditional medicine [Dip. Preventive Medicine (Thai traditional medicine)].

Establishment of Thai Traditional Medical Council

Previously, the regulation of the practice of TTM and applied TTM practitioners were under the Practice of the Art of Healing Act. Later, *Thai Traditional Medicine Profession Act B.E. 2556* (2013) was promulgated on 1 February 2013, as a result, in the near future Thai Traditional Medical Council and its Commission will soon be established to regulate the practice of TTM doctors. In the meantime, Director Generals and administrators of DTAM and Department of Health Service Support are now serving as members in the committee and subcommittees set up under suspending clause of the Act to organize the election of Commission of the TTM Council, organize licensing examination for eligible persons, and accredit academic institutions and schools teaching TTM and applied TTM.

4.3. Activities to improve the quality of herbal medicinal products and promote public use of the products

4.3.1. *Develop GMP production units in some community and general hospitals*. Several community and general hospitals have herbal medicine production units. DTAM has promoted and financially supported the development of GMP-certified production units in such hospitals. So far, seven are now GMP-certified, six are expected to be certified in 2014, and 34 hospitals are in the process of upgrading their production standard towards GMP,

4.3.2. *Develop at least 5 champion herbal products* as leading products of the country,

4.3.3. Provide research grant to researchers in phytochemistry and pharmacognosy to support the *preparation of more monographs of materia medica in the Thai*

Herbal Pharmacopoeia, to be used as the national standard of herbal raw materials for the production of quality herbal medicines,

4.3.4. At the upstream level, establish sites for the production of young herbal plants for propagation and distribution, especially those with high demand or endangered species, and establish post-harvest handling and processing facilities,

4.3.5. Promote the use of traditional/herbal medicine in NLEM by distributing the medicines to public hospitals via Government Pharmaceutical Organization procurement channel,

4.3.6. Promote public utilization of herbal medicines instead of modern drugs for common minor symptoms by introducing "*Herbal Medicine Box*" via the media and press and via provincial health offices,

4.3.7. To promote the use of traditional/herbal medicines, DTAM and the Food and Drug Administration (FDA) are now collaborating on the following issues :

- Select more items of herbal medicines into the NLEM,
- Decentralize the licensing of herbal medicine from Bureau of Drug Control, FDA to provincial health offices,
- Revise the manufacturing standards of herbal medicines in public hospitals,
- Revise the rules and regulations on the statement of indications (health benefit) of traditional medicines on the label or product insert of the products.

4.4. Knowledge generation and management activities

4.4.1. Prepare and publish "Dictionary of Thai traditional medicine and pharmacy" to give definitions on traditional TTM terminologies using modern day language,

4.4.2. Prepare and publish "Monographs of Selected Thai Materia Medica",

4.4.3. Conduct in-house researches and/or provide grants and collaborate with universities to conduct :

- pre-clinical and clinical researches on traditional and herbal medicines and traditional therapies,
- documentary researches on classical textbooks and scriptures, and
- social science research on Thai traditional medicine, indigenous medicine, and folk healers,

4.4.4. Develop Thai Traditional Medicine Digital Knowledge (TTDK) Databases on Thai herbs, research publications, classical TTM textbooks and scriptures, vocabularies, etc.

5. Protection of traditional medical knowledge and related genetic resources

The issues of the protection of traditional knowledge (TK) and related genetic resources (GR) and the equitable sharing of the benefits derived from TK and GR have been in hot debate between developed countries that have technologies and developing countries that have TK and GR in both CBD (Convention on Biodiversity) and WIPO (World Intellectual Property Organization) arenas. Thailand was at the forefront to make the first move to draft and issue a *sui generis* law to protect her TK and GR called "***the Protection and Promotion of Thai Traditional Medicine Knowledge Act B.E. 2542***" in 1999. Under the Act, DTAM serves as the secretariat office of the Committee on the Protection and Promotion of Thai Traditional Medicine.

The Act serves as a measure to promote public awareness of the value of Thai traditional medicine knowledge and herbs as well as to increase public participation on the conservation, development and sustainable use of TK and GR related to TK. In short, the Act protects

- the traditional medicine formulae, texts on TTM,
- Thai herbs that are valuable for research, or have economic significance or are endangered or threatened species, and
- Habitat of the herbs.

DTAM is responsible for the compilation of the information on TTM knowledge including TTM formulae and texts on TTM all over the country and the preparation of registers of such information using information technology for data entering, retrieval and data networking

TTM knowledge shall be separated in 3 types, namely : -

1. The national formulae of traditional Thai drugs and the national texts on TTM.
2. The general formulae of traditional Thai drugs and the general texts on TTM.
3. The personal formulae of traditional Thai drugs and the personal texts on TTM.

Regarding the protection of medicinal plants, the Minister of Public Health, with the advice of the Committee on the Protection and Promotion of TTM (the Committee), shall have the power to specify the categories, characteristic, types, and names of herbs that are valuable for study and research, or have economic significance, or are endangered species, as controlled herbs. Currently, the group of plants called "Kwao" in Thai has been protected under this Act.

In case any area in which the herbs originated have natural ecological system or biological diversity that may be destroyed or may be easily affected by humans or the entry into the area for use of herbs has caused risk to extinction or degradation or reduction of species of herbs, or the state aims at increasing public involvement in the management, development and making use of herbs in the area, and the area has not been notified as conservation area, the Minister, with the advice of the Committee, has the power to

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

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Abstracts

The aim of this research was to investigate antibody titer effect in mice treated with crocatin and deacetyl crocatin 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 crocatin and deacetyl crocatin 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., crocatin and deacetyl crocatin, 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 imunommodulatory activity was also reported (Hartini *et al.*, 2013a). In general, plants that have imunommodulatory 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 (crocatin and deacetyl crocatin) activate the phagocytic function (Hartini, *et al*., 2013b). This research aim to know the effect of crocatin and deacetyl crocatin 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. Crocatin and deacetyl crocatin 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 Commision for pra-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 treathment group, received 10 mg/kg BW deacetyl crocatin while the control group received 0.7 ml of 1% sodium carboxy methyl cellulose as solvent control, per oral for 14 days. On 15^{th} day (=day 0), 0.2 ml *L. monocytogenes* containing $5x10^3$ cfu/ml are injected intraperioneally 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 crocatin, Group B, received 5 mg/kg BW crocatin, Group C, received 10 mg/kg BW crocatin, Group D, received 2.5 mg/kg BW deacetyl crocatin, Group E, received 5 mg/kg BW deacetyl crocatin, Group F, received 10 mg/kg BW deacetyl crocatin, 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 15^{th} day (= day 0) and 25^{th} day 0.2 ml *L. monocytogenes* containing $5x10^3$ cfu/ml are injected intraperioneally 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 crocatin while isolate 2 was named deacetyl crocatin. The existence of an acetyl group (OCH_3) at $C_{1'}$ to distinguish crocatin of deacetyl crocatin having hydroxyl groups (OH). The chemical structure differences crocatin and deacetyl crocatin are shown in Figure 1. Croatin is *2-allyl-4-(1'-hydroxy-1'-(3 ", 4", 5 "-trimethoxyphenyl) propan-2'-yl) -3,5-dimethoxycyclohexa-3, 5-dienone and deacetyl crocatin 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 rendemen, size crocatin and deacetyl crocatin 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 croctin and deacetyl crocatin fairly simple, allowing the two compounds used as chemical markers for leaves of *Piper crocatum*. Crocatin and deacetyl crocatin can be used as a marker compound, which is a therapeutic components for *Piper crocatum*.

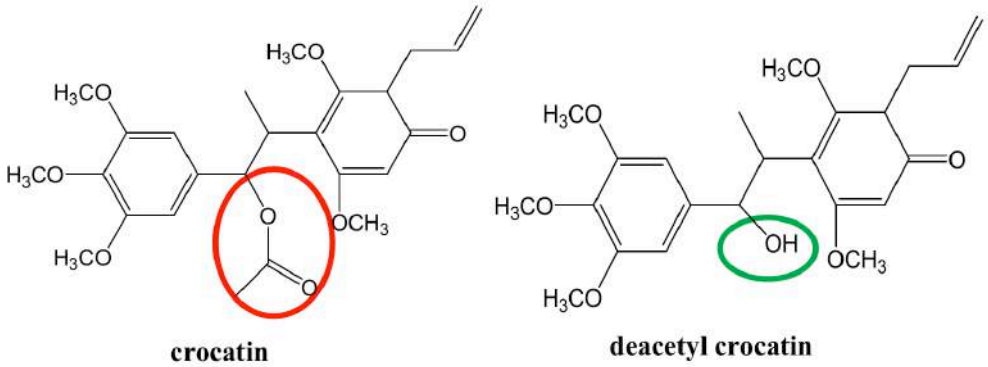

Figure 1. The chemical structure differences between crocatin and deacetyl crocatin.

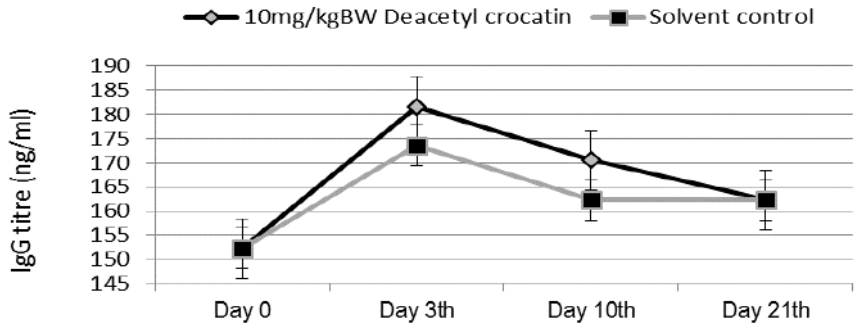

Figure 2. IgG titer levels after the mice were infected by *L. monocytogenes*.

Figure 1 shows the result of preliminary study. In this study, IgG titers of mice treated with 10 mg/kgBW deacetyl crocatin 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 crocatin 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 crocatin 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 crocatin and deacetyl crocatin ie : 2,5; 5; and 10 mg/kgBW. The result of main study can see on Figure 3.

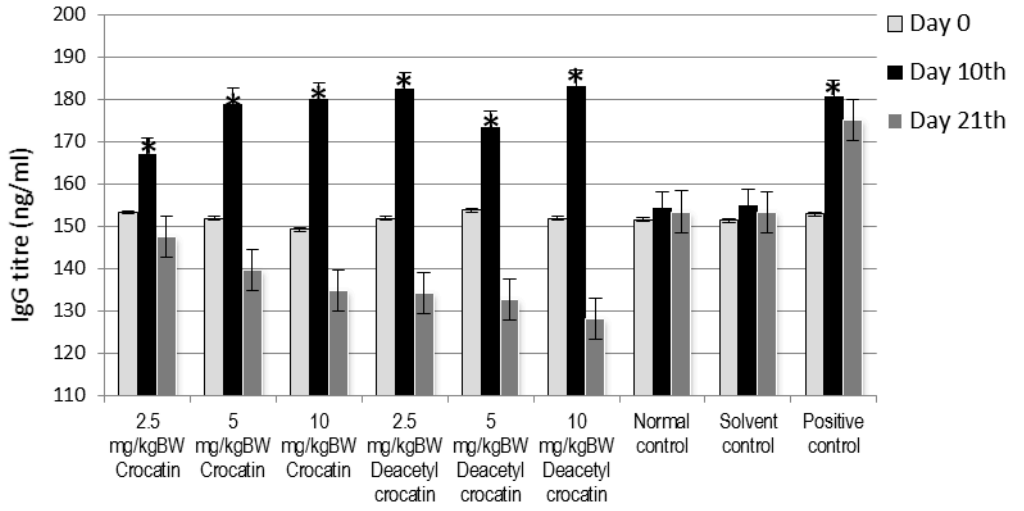

Figure 3. The effect of crocatin and deacetyl crocatin against IgG titers in mice after twice infection with *L. monocytogenes*. Values are mean ± SD of 3 replicate, *denotes significant difference ($P < 0.05$) to the normal control and the solvent control.

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 intracellulair 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 cellulair 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 $C_{1'}$ 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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EFFECT OF THE WATER FRACTION OF *Solanum torvum* SWARTZ FRUIT ON EXPERIMENTALLY INCREASED PROSTATE SPECIFIC ANTIGEN (PSA) IN WISTAR RATS

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Abstracts

PSA serum levels are abnormally elevated in patients with prostate cancer, BPH and patients with prostate inflammatory conditions. The effect of the administration of test extracts and finasteride along with testosterone on the PSA level in rats is an indication of the hypertrophy of the prostate induced by testosterone. The objective of this present study was to determine the effect caused by the water fraction of *Solanum torvum* fruit on experimentally increased PSA in Wistar rats.We included 30 adult male Wistar rats, of 6 groups (normal control, testosterone, finasteride, and water fraction dose 30; 60; 120 mg/kg p.o.) with each group comprising of 5 animals each. Testosteron (3 mg/kg s.c.) was administered to the rats along with the test fractions for a period of 28 days. Finasteride was used as positive control (1 mg/kg p.o.). After the period of administration, the rats were sacrificed, blood samples were taken from the heart apex in other to test for the PSA values. The normal PSA level in vehicle-treated group was found to be 0.225±0.081 ng/ml. This level increased to 1.668±0.208 ng/ml in testosteron-treated group. Finasteride treated group show a decrease in PSA level to 0.965±0.156 ng/ml. Water fraction in dose of 30, 60 and 120 mg/kg bwp.o. showed levels of 1.434±0.216; 1.341±0.315 and 0.909±0.194 ng/ml respectively. The PSA levels decreased in the water fraction of *Solanum torvum* fruit-treated groups, indicating their usefulness in the treatment of benign prostatic hyperplasia.

Keywords : *Solanum torvum* Swartz, Prostate Specific Antigen, Benign Prostatic Hyperplasia, water fraction, finasteride

INTRODUCTION

Benign Prostatic Hyperplasia (BPH) is a non-malignat growth and uncontrolled cells andstroma of the prostate gland that causes urinary difficulties, become the most frequent cause of lower urinary tract symptoms (LUTS) in men over 50 years, its frequency increases with age (Molina *et al*., 2007). Biochemically, BPH is considered to be an imbalance between androgen/estrogen (Untergasser *et al*., 2005), overexpression of stromal and epithelial growth factors, cytokines, and steroid hormones (Sciarra *et al*., 2008).Pathologically, BPH is characterized by hyperplastic epithelial and stromal growth that emerge into numerous microscopic and macroscopic nodules in the prostate gland (Ho and Habib, 2011), tissue remodeling in the aging prostate (Tsurusaki *et al*., 2003), stem cell defects (Lin &Chang, 1997), hypoxia (Berger *et al*., 2003), and chronic inflammation (Kramer *et al*., 2007; Nickel *et al*., 2007).

The principal prostatic androgen is dihydrotestosterone, which is formed by the steroid enzyme 5α-reductase from its substrate testosterone (Russell &Wilson, 1994). 5α-reductase is a membrane-bound NADPH-dependent enzyme that catalyses the reduction of testosterone to the more potent androgen, dihydrotestosterone. The effect of dihydrotestosterone is purely androgenic in that, unlike testosterone, it cannot be transformed into oestrogen. Two isoforms of 5α-reductase have been cloned, expressed and characterised (types 1 and 2) that display different tissue expression patterns, enzyme kinetic parameters and chromosomal localisation (Jenkins *et al*., 1991). Both isozymes are overexpressed in BPH tissue (Iehle *et al*., 1999). Because BPH therapy can reduce dihydrotestosterone levels by blocking its conversion from testosterone, 5α-reductase inhibitors could be useful in the treatment (Bartsch *et al*., 2002).

The prostate gland produces unique tissue-specifi c proteases that include prostate-specific membrane antigen (Ghosh & Heston, 2004) and members of the kallikrein family of serine proteases, including prostate specific antigen (PSA) (Lilja, 2003; Ménez *et al*, 2008). PSA are produced by epithelial cells within BPH tissue and by prostate cancer cells (Pound, 1999). BPH can increase PSA levels, thereby increasing the likelihood of detecting unsuspected prostate cancer (Meigs *et al*., 1996). PSA serum levels are abnormally elevated in patients with prostate cancer, BPH and patients with prostate inflammatory conditions (Catalona *et al*., 1995).

The present generation of medical treatments including a-blockers and 5α-reductase inhibitors are essentially monotherapies. Additionally, they all exhibit a variety of side-effects forcing many patients to consider alternatives such as the use of plant-derived medication. On the whole, phytotherapeutic drugs demonstrate remarkably benign side-effects and are virtually free of deleterious effects on sexual function (Agbabiaka *et al*., 2009).

Solanum torvum Swartz is one of theplant speciesthat are wide spread in almost all regions of Indonesia, and is widely used as a traditional medicinein the community (Sirait, 2009). The use of fruit for prostate disordersare widely used by the people of Indonesia. Zuhud (2012). reportedthat *S.torvum* fruitas anti-prostate disorders has beenempiricallyproven to be effective through his personal experience. Also based on the experience ofthe village communities since the first, consumption of unripe fruit everyday can overcome prostate disorders.The purpose of this study was to determine the effect caused by the water fraction of *Solanum torvum* fruit on experimentally increased PSA in Wistar rats.

MATERIALS AND METHODS

Chemicals

Testosterone propionate (Sigma Aldrich), finasteride (Sigma Aldrich), Total PSA ELISA kit (DRG International Inc., USA), olive oil (Bratachem), fruits of *Solanum torvum* were gotten from Bandung. Ethanol, ethyl acetate, n-hexane (Bratachem).

Preparation of water fraction of *S. torvum*

Powder of *Solanum torvum* fruit macerated with ethanol, the ethanol extract obtained was added with hot water, filtered. The filtrate then carried out with liquid-liquid extraction with n-hexane, was obtained n-hexane fraction and water fraction. Water fraction then carried out with liquid-liquid extraction with ethyl acetate, was obtained ethyl acetate fraction and water fraction.

Animal Grouping

Management adult male Wistar rats, weighing between 152g-218g were obtained from the animal house of the Department of Pharmacology-Clinical Pharmacy, School of Pharmacy, Institute Teknologi Bandung. The rats were housed in a controlled environment, where they had access to food, water, and air. These rats were acclimated in such an environment for the first week. Six groups containing five rats per group were created for this study. Hyperplasia was induced by subcutaneous administration of testosterone (3 mg/kg) for 28 days in all the groups except the vehicle-treated group. Rats were treated with vehicle or finasteride (1 mg/kg, p.o.), water fraction (30, 60 or 120 mg/kg, p.o.), before administration of olive oil (s.c.) or testosterone (3 mg/kg, s.c).

Measurement of PSA

Prostate-specific antigen levels were measured for individual rats of each group to find the extent of hyperplasia induced in the prostate by testosterone treatment. For this purpose, PSA ELISA kit was utilised. The PSA ELISA kit is intended for the quantitative determination of total PSA. This kit was obtained from DRG International Inc., USA. ELISA were performed according to the manufacturer's instructions. The color intensity was determined in the microtiter plate spectrophotometer at 620 nm. Calibration curves were constructed for each assay by plotting absorbance versus the concentration of each calibrator. The PSA concentration of samples was then read from the calibration curve.

Statistical analysis

Calculation was performed using the SPSS statistical package. Data analysis was performed by one-way ANOVA followed by Tukey post Hoc. All results was shown as mean ± SD. In all comparisons, $P < 0.05$ was considered as the criterion of significance.

RESULT AND DISCUSSION

The PSA serum levels are abnormally elevated in patients with prostate cancer, BPH and patients with prostate inflammatory conditions (Nilsson, 1997). The effect of the administration of test extracts and finasteride along with testosterone on the PSA level in rats

is an indication of the hypertrophy of the prostate induced by testosterone. This parameter was measured in the serum of the test animals of various groups using PSA ELISA kit following the procedure supplied with the kit. As shown in table 1 and figure 1, the normal PSA level in vehicle-treated group was found to be 0.255 ± 0.081 ng/ml. This level increased to 1.668 ± 0.208 ng/ml in testosterone-treated group. Finasteride-treated group showed a decrease in PSA level to 0.965 ± 0.156 ng/ml (P< 0.05 compared to testosterone-treated group). Water fraction in dose of 30, 60 and 120 mg/kg bw p.o. showed levels of 1.434 ± 0.216 (P> 0.05); 1.341 ± 0.315 (P> 0.05) and 0.909 ± 0.194 ng/ml (P< 0.05) respectively, which indicate the protective effects of water fraction on testosterone-induced hyperplasia. The decreases observed were significant compared to testosterone-treated group. These observations indicate that water fraction in dose 120 mg/kg was more effective than dose 30 and 60 mg/kg in counteracting testosterone-induced hyperplasia.

Table 8.Mean prostate-specific antigen (PSA) level of various group

Treatment	PSA level (ng/ml)
Blank (vehicle only)	0.225±0.081
Testosterone (3 mg/kg s.c.)	1.668±0.208
Finasteride (1 mg/kg p.o.) + T	0.965±0.156*
WF 30 + T	1.434±0.216
WF 60 + T	1.341±0.315
WF 120 + T	0.909 ±0.194*

Values are given as Means ± S.D. n = 5. One way ANOVA followed by Tukey Test. For each group, *Values are significantly different from testosterone-treated group at p<0.05.T = testosteron propionate 3 mg/kg s.c.WF 30, WF 60, WF 120 = water fraction of Solanum torvum Swartz (30, 60, 120 mg/kg p.o. respectively.

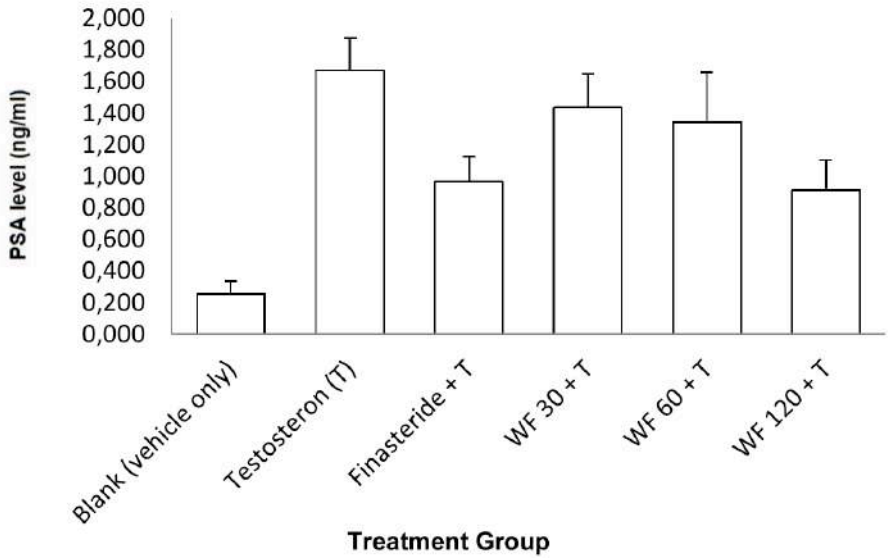

Figure 1. Mean prostate-specific antigen (PSA) level of various group.

The effects of testosterone and dihydrotestosterone on prostatic growth in rodents have previously been documented and used to assess the effects of drugs used for prostatic hyperplasia treatment (Paubert-Braquet *et al*., 1996; Marandola*et al*., 1997).PSA levels were measured at the end of the study i.e. on 28th day. PSA is a protein produced by the cells of the prostate gland. The PSA test measures the level of PSA in the blood. PSA serum levels are abnormally elevated in patients with prostate cancer, BPH and patients with prostate inflammatory conditions. If a decrease in PSA levels is observed, it can be assessed that the test sample in question is having protective effects on the inflammatory conditions and hypertrophy of the prostate induced by testosterone. Testosterone treatment increased the PSA levels, which is an indication of hyperplasia, whereas finasteride reduced the PSA levels significantly suggesting its protective effects. The water fraction of *Solanum torvum* dose 120 mg/kg significantly reduced the PSA levels which are an indication of its 5α-reductase activity and efficacy in the treatment of prostatic hyperplasia.

CONCLUSION

The water fraction of *Solanum torvum* fruit dose 120 mg/kg significantly reduced PSA level on experimentally increased PSA in Wistar rats.

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