UMY UNIVERSITAS MUHAMMADIYAH YOGYAKARTA
Unggul & Islami

SCHOOL OF PHARMACY
FACULTY OF MEDICINE AND HEALTH SCIENCES

RISTEKDIKTI

ISCC

IKATAN APOTEKER INDONESIA IAI

PROCEEDING

1ST INTERNATIONAL CONFERENCE ON PHARMACEUTICAL UPDATES

"Trending on Chemopreventive Agent : Cancer Immunology & Cancer Metabolism"

SCHOOL OF PHARMACY
Faculty of Medicine and Health Sciences
Universitas Muhammadiyah Yogyakarta

1st International Conference on Pharmaceutical Update
Universitas Muhammadiyah Yogyakarta
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1st ICPU International Conference on Pharmaceuticals Update

Trending on Chemopreventive Agents: Cancer Immunology and Cancer Metabolism

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PREFACE

Alhamdulillah, thanks to Allah SWT Who has given us health and mercy. Greeting and Prayers we always send for our prophet Muhammad SAW. In order to demonstrate the synergy of the collaboration between the researcher in the cancer chemoprevention, School of Pharmacy Universitas Muhammadiyah Yogyakarta in cooperation with Indonesian Society of Cancer Chemoprevention (ISCC) held The 1st International Conference on Pharmaceutical Updates (ICPU) Universitas Muhammadiyah Yogyakarta and The 9th Annual Symposium ISCC.

The theme discussed in the conference this year is Trending on Chemopreventive Agents: Cancer Immunology and Cancer Metabolism. We hope this conference can enhance the development of cancer chemoprevention researches in Indonesia, disseminate the results of the research, news, and ideas on the development of cancer chemoprevention, mobilize, build network strength and cooperation between individuals and institutions for the better value of the cancer chemoprevention Indonesia.

Best Regards,

Committee

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SPEECH

RECTOR
UNIVERSITAS MUHAMMADIYAH YOGYAKARTA

In an increasingly global world, increasing human interactions between continents, increasing world population and environmental degradation are major issues in the 21-century. Decreasing environmental quality causes health problems that can be caused by the risk of disease transmission and increased resistance to germs (micro-organisms). This causes human health problems to become more complex, and therefore a new approach to the problem of medicine is needed.

Provision of medicinal ingredients can be carried out through isolation and synthesis techniques from various medicinal sources including plant tissue, animal tissue, microbial culture technology, and even by developing biotechnology techniques to produce medicinal ingredients in a faster time. Based on the connection between drug structure and biochemical reactions, the search for new drug ingredients can be done through the concept of medical chemistry and molecular pharmacology.

1st International Conference on Pharmaceutical Updates in collaboration with the Indonesian Society for Cancer Chemoprevention (ISCC) is the first scientific forum in the field of medicine that tries to discuss the current issues of the problem of medicine. I, as the Rector of the Universitas Muhammadiyah Yogyakarta, hope that this conference will produce the new academic conclusions in developing the drug ingredients and the production of drug preparations.

Finally, I congratulated and success to the Department of Pharmacy, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta for this smart initiative. Thank You.

Best regards,

Rector

Dr.Ir. Gunawan Budiyanto,IPM.

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SPEECH

DEAN
FACULTY OF MEDICINE AND HEALTH SCINCES
UNIVERSITAS MUHAMMADIYAH YOGYAKARTA

Dear Colleagues

It is my privilege and great pleasure to warmly welcome to the 1st International Conference on Pharmaceutical Updates & 9th Annual Symposium Indonesian Society for Cancer Chemoprevention that is taking place in Yogyakarta, Indonesia. I believe that all of you have settled in and looking forward to the next three days attending the scientific session and exhibition. I am sure that it will also to be a great opportunity for all you to gain the number of friend and colleagues to build up the future scientific collaboration.

The 1st International Conference on Pharmaceutical Updates & 9th Annual Symposium Indonesian Society for Cancer Chemoprevention have been put a great effort toward this event. They are also working with varies stakeholder, including RISTEK DIKTI and Ikatan Apoteker Indonesia members, to manifest the excellence scientific program where the number of invited speakers will speak on cutting edge science in "Trending on Chemopreventive Agent: Cancer Immunology & Cancer Metabolism". In addition, this conference will also be platform for delegates to showcase their research in the form of oral and poster presentation.

I would like to express my gratitude to our sponsor and exhibitors, and last but not least to conference committee for all their effort in making this event a successfully conference.

Thank you for attending our invitation to join us in Yogyakarta. I am sure all of you will have a memorable and enjoyable conference, please enjoy the beautiful of Yogyakarta together with friends and colleagues.

Best Regards,

Dean

Dr. dr. Wiwik Kusumawati, M.Kes.

SPEECH

CHAIRMAN OF INDONESIAN SOCIETY FOR CANCER CHEMOPREVENTION (ISCC)

Assalaamu'alaikum wr wb.
Dear colleagues,

First of all, I would like to express my gratitude to the Rector of Universitas Muhammadiyah Yogyakarta and all of the staff members of the Departement of Pharmacy UMY for this great effort and opportunity to be the host of this conference. On behalf of the chairman of Indonesian Society for Cancer Chemoprevention (ISCC), it is my pleasure to welcome all the participant to this conference, the first International Conference of Pharmaceutical Update and the 9nt Annual Conference of ISCC. Thank you for your great support and participation in this conference to attend and share your updated achievement in the field of Pharmaceutical sciences and cancer chemoprevention. This is our concern commitment to develop together effectively the newest science and technology to combat cancer under chemoprevention paradigm.

This year, our annual conference is held in collaboration with the Departement of Pharmacy, Universitas Muhammadiyah Yogyakarta (UMY) with the theme of "Trending of Chemopreventive agents: Cancer Immunology and Cancer Metabolism" with the more targeted therapy in cancer treatment with BNCT. These three topics are become the hot issues in cancer treatment in the world. Fortunately, the invited speakers, covering these topics and the related fields already prepared the updated recent information to be shared in this occasion. We hope that all of the newest knowledge presented in this conference would be useful for our comprehensive understanding to improve our profession, academic and research activities. Moreover, our participation in this event surely will increase and strengthen our networking and capacity building in each of our institution. We would like to thank the Minister of RISTEKDIKTI, the Rector of UMY, the Head of the Departement of Pharmacy UMY, Nara Institute of Science and Technology (NAIST)-Japan, BNCT research Center –Osaka Prefecture University-Japan, The Speakers, the committee and all of the participant. Thank you very much for your kind attention,

Wassalaamu'alaikum wr wb.

Chairman
Indonesian Society for Cancer Chemoprevention

Prof. Dr. Edy Meiyanto, M.Si., Apt.

SPEECH

CHAIRMAN OF 1ST ICPU

Good Morning, Assalammualaikum wr.wb

First of all, let us praise to Allah SWT who has given us health and opportunity to attend the 1st International Conference on Pharmaceutical Updates at Universitas Muhammadiyah Yogyakarta today. Greeting and Prayers we always send for our prophet Muhammad SAW.

On behalf of the Committee, I would like to say thank you to :

The honourable keynote speaker **dra. Engko Sosialine Magdalena, M.Bio, Apt** from Indonesia Ministry of Health Representative; **Dr. Ir. Gunawan Budiyanto, M.P** rector of Universitas Muhammadiyah Yogyakarta (UMY); and also **Dr.dr. Wiwik Kusumawati, M.Kes**, Dean of Medical Faculty and Health Sciences UMY for your speech to open the conference today.

The honourable all the invited speakers **Prof. Yashwant V Pathak** from United State of America, **Prof. Jun Ya Kato** and **Prof. Mitsunori Kirihata** from Japan, **Dr.Suthan Chanthawong** from Thailand, **Prof. Dr. Ibrahim Jantan, MSc., PhD** from Malaysia, **Dr. James Henshall** from Australia, **Prof. Katja Taxis** from Netherland, and **Sabtanti Harimurti, PhD, Apt** from Indonesia for your willingness to come and share your experience in accordance with the current update about Cancer Immunology and Metabolism

The Prestigious, Indonesian Society for Cancer Chemoprevention, for trusting us from the School of Pharmacy, Faculty of Medicine and Health Sciences, UMY to be your partner in conducting today's International Conference. We are so grateful to receive this opportunity and work along with you.

The honourable, our special guest and all participants for the enthusiasm and active involvement in today's conference. I'm feeling glad to report that we have 108 registered participants came from Yaman, Sudan, Malaysia, Thailand, and Indonesia. Thank you so much for the trust and participation.

Last but not least, I would like to send my deepest gratitude to all the committees who have given all the hard work to make this first International Conference happened. There is no word which can describe how thankful I am to have such a wonderful team like you. I am truly proud to work with you all.

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This conference is aimed to provide you essential information about the current invention related to patient care, drugs development and pharmaceutical technology of cancer and chemoprevention. Hopefully, this conference may facilitate us to have some information exchange as well as collaboration initiative between Indonesia and overseas researchers. I am proudly announce that this conference will be conducted in every two years, and the next International Conference on Pharmaceutical Updates will be held in 2020.

In my closing speech, I would like to tell you that we have Yogyakarta with so many tourist spots that you can enjoy by yourself or you can also join us on our City Tour Agenda along with our Social Activity which will be held in Kraton Yogyakarta, Tamansari, Masjid Agung, and Malioboro.

Finally, I wish you enjoy the conference as well as Yogyakarta.

Wassalammualaikum, wr. wb.

Yogyakarta, 3rd October 2018
Chairman of 1st iCPU

Pramitha Esha ND, M.Sc, Apt

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Interconnection Between Carboxymethyl Chitosan and Anticancer A Mast Cell Study

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Abstract

Activation of mastocytes can produce cytokines such as TNF and tumor growth factor (TGF) -β1. The expression of all the genes above is mainly regulated by the transcription factor NF-kB which is activated by free radicals. The purpose of this study was to examine the relationship between carboxymethyl chitosan and anticancer in a mast cell study. The inhibition of active mastocytes can eliminate the process of cancer, so it needs KMK which can suppress cancer through H2O2. In vivo, ovalbumin-censored mice, H2O2 levels reached 271.93 ng/ml compared with negative controls around 213.45 ng/ml. After being given carboxymethyl chitosan H2O2 levels to 220.85 ng/ml. Reducing levels of H2O2 mastocytes is an effect of KMK. Thus the relationship between KMK and anticancer is a substance or compound that can inhibit free radical activity through active groups of KMK.

Keywords: Tumors, H_2O_2, Mastocytes, carboxymethyl chitosan, anticancer.

PRELIMINARY

Mastocytes (mast cell) are cells that are only found in tissues associated with blood vessels (Baratawidjaja, 2000). Mastocytes are known to have a very broad biological spectrum such as immediate hypersensitivity, delayed hypersensitivity, immune regulation, fibrosis, myelopoiesis, angiogenesis, tissue repair, bronchial contriction, cytotoxicity and intestinal hypermotility (Widjajanto, 2007). Activation of mastocytes can produce cytokines such as TNF-α, IL-1, IL-4, IL-6, IL-8, IL-13 and tumor growth factor (TGF) -β1 (Plaut et al 1989); Wodnar-filipowicz et al 1989; Bradding et al 1993). Some pro-inflammatory gene products have been identified as having an important role in suppressing apoptosis, proliferation, angiogenesis, invasion, and metastasis. Among the products of the gene is TNF-α, and members of their superfamily include IL-6, IL-8, IL-18, chemokine, MMP-9, VEGF, COX-2, and 5-LOX. The expression of all the genes above is mainly regulated by NF-kB transcription factors which are constitutively active in most tumors and induced by carcinogens (cigarette smoke), promoter tumors, oncogenic viral proteins, chemotherapy agents, and gamma irradiation (Aggarwal et al 2006). Cancer causes are free radicals.

Free radicals are atoms or molecules that have one unpaired electron. These unpaired electrons have the tendency to form pairs by drawing electrons from other compounds so that new radicals are formed that are very reactive (Halliwel & Gutteridge, 1999). Free radicals can come from metabolic processes in the body and can also come from the environment.

Oxidative free radical chain reactions can damage cell-forming macromolecules such as proteins, carbohydrates, lipids and deoxyribonucleic acid (DNA), which results in cells becoming damaged, dead, or mutated (Januar et al, 2004). Oxidative stress and oxidative damage are products of free radicals. The body has a defense system in the form of antioxidants, but if the free radicals are more dominant and accumulate in the tissues or inside cells, the body's homeostasis through regulation between proliferation and apoptosis cannot be maintained.

Therefore, the availability of natural compounds is highly expected as a deterrent to free radicals, among others, are natural ingredients from the sea. The marine environment is a natural resource rich in secondary metabolic materials, many of which have chemical structures that are not found in terrestrial/terrestrial environments (Ibrahim, 2010). One of them is carboxymethyl chitosan.

Carboxymethyl chitosan (KMK) is a chitosan derivative through carboxymethylation. Chitosan is one of the aquatic natural resources that has biological activity. This compound is an unbranched carbohydrate polymer, containing 2-amino-2-deoxy-D-glucose with β (1-4) glycosidic bonds obtained from shrimp or crab skin through chitin deproteinization, demineralization, colorization, and deacetylation. KMK has the potential to be anticancer through inhibition of hydrogen peroxide (Ibrahim, et al 2018).

In connection with the above, research has been conducted on inhibition of KMK to increase hydrogen peroxide after being given the trigger of free radicals. The results of this study are expected to provide an overview of how the relationship between carboxymethyl chitosan and anticancer.

MATERIALS AND METHOD

A. Research Procedure.

This study was an in vivo study on mesentery of female Wistar rats in which rats were induced with ovalbumin and carboxymethyl chitosan. Next, it is dissected through anesthesia and mesenteric tissue is taken. After being given toluidine blue, mastocytes cells were observed under a microscope with 400 x magnification to determine the active cell mastocytes. Then protein isolation was carried out on the mesenteric tissue using Colorimetric hydrogen peroxide Kit (catalog No. 907-15) (Ibrahim et al 2009). The resulting data is analyzed in the description.

RESULT AND DISCUSSION

The results of the study showed that mastocyte cells originating from the white rat mesentery tissue could be identified using toluidine blue staining. Mastocytes have granules that contain a number of chemical mediators/cytokines, if activated cells will release these cytokines including free radicals to trigger cancer.

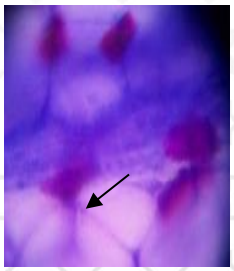

Figure 1. Perivascular mastocyte staining results with toluidine blue through microscope observation with 400 x magnification. Mastocyte cells can be seen clearly, active mastocytes, with black arrows.

The results showed that the average value of H2O2 in Wistar rats that were not given ovalbumin or acted as negative controls was 213.45 ng/ml ± 0.90. The rat's given ovalbumin were then given carboxymethyl chitosan at a dose of 0.50 mg at 220.85 ng/ml ± 4.31, and the rats given ovalbumin as a positive control were 271.93 ng/ml ± 2.42 (Table. 1).

Table 1. The average yield of H_2O_2 ng/ml

No	Free Variable	Varibel bound ng/ml
1	Negative Control	213,45
2	KMK	220,85
3	Positive Control	271,93

DISCUSSION

One of the inflammatory responses is a cellular response, so there is acute inflammation and chronic inflammation. Chronic inflammation occurs when acute inflammation fails and antigens persist causing continuous activation and accumulation of macrophages. According to Aggarwal et al (2006), activation and accumulation of macrophages including mastocytes can cause cancer. Activation of mastocytes is caused by sensation from ovalbumin so that cross-linking between receptors and ligands causes cytokines in mastocyte cell granules to emerge including tumor-triggering genes whose expression is regulated by transcription factors NF-KB (Aggarwal et al 2006). from crosslinking between ligands and receptors found in the mastocyte cell membrane. That is why when mastocytes become active there is an increase in the amount of H2O2 value, one of the free radicals triggers from cancer. Therefore we need antioxidant compounds to avoid cancer.

Ocean waters are very rich in antioxidant compounds. One of the antioxidant compounds from marine waters is chitosan from the skin of shrimp or crab skin. Chitosan through carboxymethylation can be converted into carboxymethyl chitosan (KMK). Dwiyitno et al (2004) said that carboxymethyl chitosan (KMK) is chitosan soluble in water, non-toxic and biodegradable and biocompatible. Carboxymethylation is an esterification process of chitosan alkalis with monocloroacetic acid, where the carboxymethyl group (CH2COO-) of monocloroacetic acid substitutes hydroxyl groups (OH-) or amine groups (NH2) in chitosan to form carboxymethyl chitosan (KMK) (Bader & Birkholz, 1997)

KMK is widely used in the pharmaceutical and health fields such as hydrogel, cholesterol reducer, antibacterial and antioxidant (Davies et al 1988; Muzzarelli et al, 1997; Zhai et al, 2003; Yang et al 2005). KMK as water-soluble chitosan can significantly block the secretion of TNF-a and IL-8 stimulated with calcium ionophore in the human mastosit line (HMC-1) (Kim et al 2004). Whereas according to Seo et al (2003), water-soluble chitosan can inhibit NF-KB induced by deferoxamine in the human mastosit line. According to Chen et al (2008), water-soluble chitosan inhibits PKC phosphorylation and NF-KB activity induced by allergen-stimulated MDM.

Decreased levels of H2O2 by carboxymethyl chitosan because KMK has amino and carboxyl groups. This active group can inhibit free radical activity. Hall & Cuppet (1997) said that antioxidant compounds in neutralizing a free radical can be through 3 reaction mechanisms, namely breaking free chains, metal plating, and single oxygen quenching.

According to Halliwell & Gutteridge (1999), these amino groups and carboxyl groups can function to neutralize free radicals through free chain breaking. Proton or hydrogen donors from hydroxyl groups or amino groups from chitosan soluble in water will break free radical chains (superoxide anions, O2- and hydrogen peroxide, H2O2) to form non-radical compounds (Yang et al, 2005), so the cause of cancer that is, the presence of free radicals can be avoided. Thus the relationship between KMK and anticancer is a substance or compound that can inhibit free radical activity through active groups of KMK.

CONCLUSION

Based on the results and discussion concluded that:

1. Mastocytes can be identified with toluidine blue staining.
2. The interconnection between carboxymethyl chitosan and anticancer is an active group of carboxymethyl chitosan namely amino groups and carboxyl groups as the mechanism of antioxidant compounds in neutralizing free radicals, especially H2O2 which can trigger cancer.

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ICPU_2019_002

Case Report of Acupuncture in Treatmeant of Pain in Bone Metastasis

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Abstract

Bone pain caused by cancer is a common complication in bone metastasis. Bone pain has the characteristic triad of pain including continuous pain, spontaneous pain and incident pain. Various mechanisms that allow bone metastasis can cause pain have been reported. These mechanisms include the possibility of local production of growth factors and cytokines. The management of the pain bone metastasis include aspects of pharmacological and non-pharmacological therapies. Acupuncture can play a role in the treatment of bone metastasis pain through modulation of various neurotransmitters and increase the production of endogenous opioids that can cause analgesic effect. This case study report the effect of acupuncture therapy to treat pain, with the goal of providing information on the possible use of acupuncture for painful bone metastasis. A male patient aged 62 years old with painful bone metastasis who received acupuncture therapy. Acupuncture was carried out twice a week for six weeks. At the end of therapy the patient noted improvement in pain. Acupuncture appears to be beneficial in treating pain of bone metastase. Owing to limited available evidence on this topic, further research is recommended.

Keywords
Bone pain, Metastases, Acupuncture

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INTRODUCTION

Cancer metastases are the spread of cancer cells out of their origin (primary site) to another place or body part. Pain in bone is a common complication of bone metastases and consists of pain trias in the form of continuous pain, spontaneous pain and incident pain. This state of pain can affect social function, quality of life and survival. Various possible mechanisms that cause bone metastatic pain it has been reported, this mechanism is the production of local growth factors and cytokines (TNFα, IL-1β, IL-6) either inducing tumors or producing tumors, tumors induce osteolysis, ion channel stimulation at nerve cell endings and direct infiltration. Periosteum stretching by tumor expansion, mechanical stress on brittle bones, nerve suppression by the tumor or direct damage to the bone is a possible mechanism responsible for metastatic bone pain (Wallace et al, 2014). Handling bone metastatic pain is still a difficult problem to overcome. Multimodal variety is used for handling these pain. Most patients will need pharmacological and non-pharmacological interventions to overcome this problem. However, metastatic bone pain is a complex pain. Side effects of analgesic drugs are often found due to the use of maximum doses for a long time (Mantyh PW et al, 2013). The National Cancer Institute (NCI) guidelines recommend acupuncture as an additional therapy for cancer pain patients, especially in patients who experience side effects from analgesia (Bao T, Mansky PJ, Tian X, 2016). Acupuncture is part of medical treatment by stimulating specific acupuncture points in the skin using needle stimuli, pressure, heat, electricity, lasers to treat various symptoms of the disease with minimal side effects. The following case report describes a patient with advanced prostate cancer who was suffering from bone pain metastase and successfully treated with acupuncture.

MATERIALS AND METHOD

This 62 years old gentleman present with localized pain on the booth knee. Pain is felt after cancer prostate surgery in May 2015. Initially the pain felt intermittent, like being pierced and the pain was felt continuously now. Pain increases when the foot is moved or changes position.Patients also complain of weakness on both legs. Treatment obtained in the form of analgesic medication, but without improvement. General conditions and physical examination are found right and left limb motor strength 4, pain in the patellar region with VAS 5. Examination routine blood hematology is within normal limits. Radiological examination in the form of a bone scan shows description of metastasis in the bones: frontal os right, vertebra C7 / Th1, Th4, Th10, Th11-Th12, L1-L3, costovertebral joint 8, os costae 8, 10 posterior right, os sacrum, left ilium os, left ischium os, os right and left femur. AP & Lateral genu: lytic lesion multiple distal metadiaphysis of the bilateral femur os, metadiafysis of bilateral tibia and fibula os, as well as lesions bilateral patellar os, especially the anterior side according to metastatic features.

Consultation is done with the anesthesiologist recommended treatment is diclofenac 50 mg, gabapentin 300 mg, tramadol 50 mg. From a radiotherapy consultation recommended

treatment is radiotherapy, the patient has one series of radiotherapy was carried out. Consultation orthopedic recommended treatment is clodronate, calcitriol

Patients started treatment for acupuncture on February 2016. The therapeutic method used was in the form of an acupuncture manual using a penetrating needling technique at acupoint ST36-ST37, BL39-BL40-LR8, BL57-BL40, Ex-B2 L2-L5. the needle used is the needle filiform acupuncture with a size of 0.25x40 mm. Acupuncture was carried out twice a week for six weeks. Duration of the treatment was 30 minutes, and the neddles were left in situ and stimulated manually every 10 minutes to increses the therapeutic effects. The measure outcome of this case report using visual analog scale (VAS) by asking patient before and after therapy in each session.

RESULT AND DISCUSSION

This paper describes the case of a 62-year-old man with a diagnosis pain et regio patella dextra sinistra et causa bone metastase. After each session of treatment, the patient noted improvement in pain. By the end of 6 weeks of treatment, his pain was under control, the intensity and frequency of pain had reduced. The patient's severity of the pain had reduced from 5/10 to 0/10 on visual analog scale at the end of treatment (table 1).

Needling into the skin with filiform needles is the most commonly used form in the stimulation of acupuncture in clinical practice. When a filiform needle is inserted into a point on the body and mechanical stimulation (manual manipulation) or electrical stimulation is given, various neural and neuroactive components are activated. The nerve bundles and activated components are scattered in the skin, muscles, and connective tissue surrounding the acupuncture needle can be defined as neural acupuncture unit (NAU) (Zhang ZJ et al, 2012).

At this time it is known that acupuncture works through three mechanisms, local, segmental and central. The local mechanism of acupuncture causes microtrauma which the release of substance P, CGRP and β-endorphin. The segmental mechanism of acupuncture points will stimulate large-diameter Aδ nerve fibers. This stimulation will be delivered to marginal cells in the spinal cord which are then passed through serotonergic fibers (5HT) to the stalk cell. These cells inhibit substance gelatinosa by the mechanism of enkephalinergik to inhibit pain stimuli. The central mechanism stimulation is transmitted from marginal cells to the ventroposterior thalamus nucleus and then projected into the cerebral cortex will stimulate serotonergic and noradrenergic fibers to the stalk cell which in turn will inhibit the substantia gelatinosa (White A, 2008). Acupuncture can regulate the endogenous release of opioids, such as β-endorphins, enkephalins, and dynorphin which will bind to the receptors μ-, δ-, and κ so as to provide analgesia (Lee AD, Hsu ES, 2014).

Table 1 Acupuncture points

Treatment Session	Acupuncture points	Duration of treatment	Manual stimulation (every 10 min)	Treatment response
1	ST36-ST37, BL39-BL40-LR8, BL57-BL40, Ex-B2 L2-L5	30 min	Yes	VAS before : 5 VAS after : 2
2	As above	30 min	Yes	VAS before : 5 VAS after : 1
3	As above	30 min	Yes	VAS before : 5 VAS after : 2
4	As above	30 min	Yes	VAS before : 4 VAS after : 1
5	As above	30 min	Yes	VAS before : 5 VAS after : 1
6	As above	30 min	Yes	VAS before : 5 VAS after : 2
7	As above	30 min	Yes	VAS before : 4 VAS after : 1
8	As above	30 min	Yes	VAS before : 4 VAS after : 1
9	As above	30 min	Yes	VAS before : 4 VAS after : 1
10	As above	30 min	Yes	VAS before : 3 VAS after : 0
11	As above	30 min	Yes	VAS before : 3 VAS after : 0
12	As above	30 min	Yes	VAS before : 3 VAS after : 0

CONCLUSION

Patients showed improvement in pain symptoms after acupuncture treatment based on reduced pain assessed by VAS. Acupuncture appears to be beneficial in treating pain of bone metastase. Owing to limited available evidence on this topic, further research is recommended.

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TEST KHEMOPREVENTIF STOCKS AND FORMULATIONS TABLET (CaCi) MANDARIN ORANGE SKIN ethanolic EXTRACT AND TEA LEAVES IN BREAST CANCER CELLS T47D METHOD IN VITRO AND IN silico

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Abstract

Breast cancer is a disease that has the highest mortality rate in Indonesia. Cancer treatment with chemotherapeutic agents cause a lot of side effects that can degrade the quality of life of patients. For it is necessary to find a relatively safe khemopreventif agents and minimal side effects. One of the plants that can be developed as a chemopreventive agent is leaf tea (Camellia sinensis) and orange peel (Citrus reticulata) were shown to inhibit the proliferation of cancer cells. This study aims to determine the cytotoxic activity of antioxidants and tea leaf extract and orange peel on T47D breast cancer cells in vitro and In Silico. This study covers the extraction process and the orange peel tea leaves by maceration method. TLC test by using Silka Gel, antioxidants using DPPH. Cytotoxic test with MTT Assay and test methods in silico using Software Autodock Vina with mendockingkan Tangeretin compound and kaempferol against a target protein Bcl-XL. Cytotoxic using ELISA Reader with T47D cells. The results of this study showed antioxidant activity in T47D breast cancer cells with IC50 values of 83.00 pg / ml. In moleculer docking, kaempferol compounds have affinity -6.4 kcal / mol, Tangeretin compounds have affinity -5.8 kcal / mol. TLC results obtained on its Rf value at 0.87 orange peel extract and extract of tea leaves 0.65. Produced cytotoxic IC50 7707.57. From the results obtained it can be concluded that the combination of ethanolic extract of orange peel and leaf tea has potential as a chemopreventive agent in breast cancer cells.

Keywords : orange peel, tea leaves, molecular docking, MTT Assay, Antioxidants

PRELIMINARY

Cancer is one of the causes of death worldwide. In 2015, approximately 8.8 million deaths are caused by cancer. Based on data from Globocan, the International Agency for Research on Cancer (IARC) in 2002, breast cancer ranks first of all cancers in women in the world. Until now, treatment of breast cancer is still on chemotherapy, radiation and surgery. In patients with breast cancer, the abnormal proteins appear to change cell behavior that is the increasing proliferation and differentiation of cells and a decrease in the ability of cell apoptosis. One protein that play a role in anti-apoptotic Bcl-XL is. To overcome these cell abnormalities, then treatment with chemical drugs.

Different types of treatment that is generating a lot of harmful side effects during treatment. Therefore, to improve the efficacy of cancer treatment and minimize side effects can be done through the development of co-agent chemotherapy drug alam.Pengembangan of materials from natural materials is feasible, some chemotherapy drugs breast cancer patients also come from wild plants. One potential natural materials as co-agent chemotherapy is leaf tea (Camellia sinensis L) and orange peel (Cirus reticulata). Tangeretin flavonoids and kaempferol in Dun Tea Orange Peel and is expected to contribute to the anticancer activity so that it can be combined with cancer drug and is expected to work synergistically as co-chemotherapy.

RESEARCH METHODS

This research was conducted at the Research Laboratory, the lab Fitomedisin, cell culture lab and Pharmaceutical Technology, University of Muhammadiyah Yogyakarta. Research dilakukanpada April2018 until July 2018.

Material

Orange peel and leaf tea, 70% ethanol, distilled water, Cells T47D, Methanol, Ethyl Acetate, Plate TLC silica gel GF254, Acetic acid glacial, Methanol, DPPH, Formic Acid, Fetal Bovine Serum (FBS) 10%, Phosphate Buffer Saline (PBS), trypsin EDTA, MTT 5 mg / ml, Sodium dodecyl sulphate (SDS) in 0.1% HCL, protein structure of Bcl-xL, Bcl-XL protein, ammonia spray, standard quersetin, starch, mg stearate, lactose, PVP.

Tool

Blender, analytical balance, aluminum foil, a set of computer, lamp UV 254 and 366 nm, oven, Laminar Air Flow, ELISA Reader, Incubator CO2, Tissue Culture Flask, Tubing conical 15 ml of sterile, Haemositometer, Cell counter and Yellow tip, Blue tip , micropipette, inverted microscope, a 96-well plate, Rotary evaporator, elemeyer, watch glass measuring cup, propipet, spatula, filter paper, sieves, mortar, Stemper, test tube

Method

Living cells would react with MTT assay reagents to form a purple color read by ELISA reader at a wavelength of 595 nm and calculated IC50, Test antioxidants with DPPH, first preparation of the solution DPPH DPPH by weighing as much as 15.8 mg powder, subsequently manufacture routine Vitamin C solution of 5 mg at a concentration series of 0.5: 01.5: 10: 15: and 20 ug / ml, then the test solution preparation to extract each weighing 50 mg with a series of concentrations of 10: 30: 50: 70 : 90: 110 pg / ml. Furthermore, the manufacture of tablet formulations perfomed with granuasi wet method, by using the phase and external phase ingredients tea leaf extract and orange peel, manihot starch, mg stearate, talc, and lactose. The first solution preparation DPPH DPPH to weigh as much as 15.8 mg powder, then the routine preparation of the solution as much as 5 mg Vitamin C with a concentration series of 0.5: 01.5: 10: 15: and 20 ug / ml, then the preparation of the solution by weighing Test each extract concentration of 50 mg with a series of 10: 30: 50: 70: 90: 110 pg / ml. Furthermore, the manufacture of tablet formulations perfomed with granuasi wet method, by using the phase and external phase ingredients tea leaf extract and orange peel, manihot starch, mg stearate, talc, and lactose. The first solution preparation DPPH DPPH to weigh as much as 15.8 mg powder, then the routine preparation of the solution as much as 5 mg Vitamin C with a concentration series of 0.5: 01.5: 10: 15: and 20 ug / ml, then the preparation of the solution by weighing Test each extract concentration of 50 mg with a series of 10: 30: 50: 70: 90: 110 pg / ml. Furthermore, the manufacture of tablet formulations perfomed with granuasi wet method, by using the phase and external phase ingredients tea leaf extract and orange peel, manihot starch, mg stearate, talc, and lactose.

RESULTS AND DISCUSSION

1. Molecular Docking Test

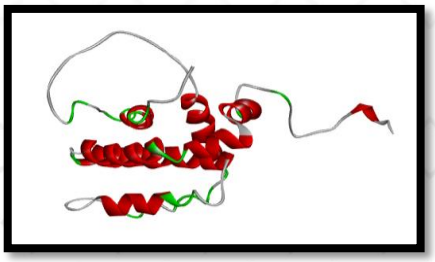


Table 1. Protein Code 1YSG

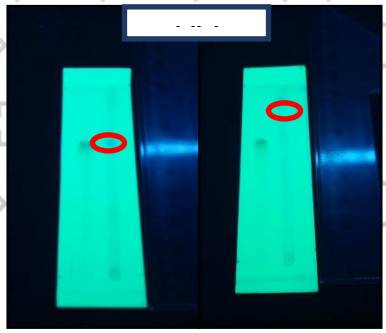
NO	Test compound	conformation	RMSD value	Docking Score (kcal / mol)
1	kaempferol	8	1,738	-6.7
2	Tangeretin	5	1,333	-4.5
3	5-Flurourasil	2	1.437	-4.9
4	doxorubicin	2	1,207	-3.6

Table 2. Protein Code 4TUH

NO	Test compound	conformation	RMSD value	Docking Score (kcal / mol)
1	kaempferol	3	1.930	-6.4
2	Tangeretin	2	1,132	-5.8
3	5-Flurourasil	5	1,513	-7,0
4	doxorubicin	5	1,901	-4.5

From the results of molecular docking test data above shows that the orange peel extract and leaf tea has the same affinity energy of chemotherapy drug 5-fu yaitui and doxorubicin. tea leaf extract and orange peel also have a great affinity value that can be used and meticulous as Ko-khemotrapi agent.

2. TEST TLC (Thin Lapias Chromatography)



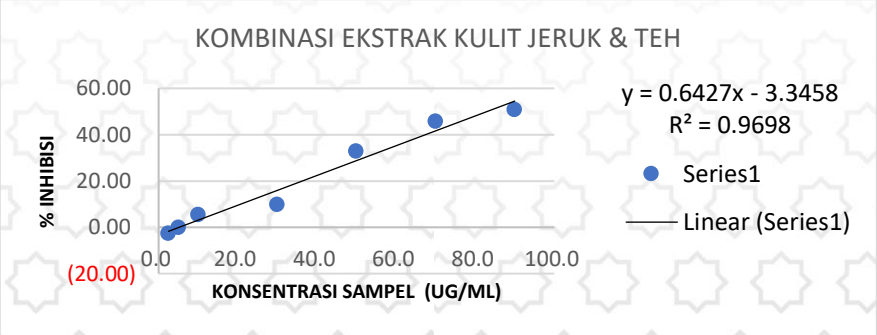
From the TLC test the UV light 254, indicates flavonoids in orange peel extract and tea leaves that has a value of Rf which is not much different from a regular comparison compound results in getting on the compound kaempferol and Tangeretin are 0.87 and 0.65.

3. Antioxidants

From the table below in this study using the 7 series concentrations of 2.5: 5.0; 10.0; 30.0; 50.0; 70.0; 90.0. At concentrations of 2.5 and 5.0 show the percent inhibition of unfavorable ie 2.61 dan0,09. But the results show the results of the linear curve.

Table. 3 IC50 value Antioxidants

Concentration ug / ml	Mean Absorbance	absorbance Blanko	Percent inhibition (%)
2.5	0.721	0,702	(2.61)
5.0	0,703	0,702	(0.09)
10.0	.664	0,702	5.46
30.0	0.633	0,702	9.87
50.0	.472	0,702	32.87
70.0	.381	0,702	45.80
90.0	0.346	0,702	50.78
IC50	**83.003**		

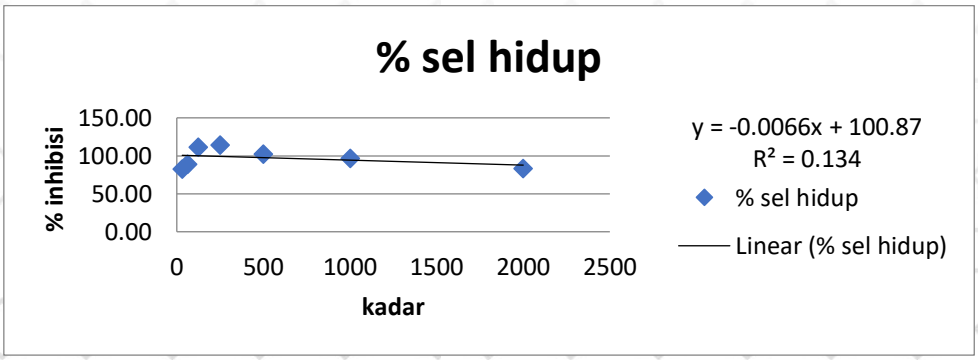


From the test data Antioxidants above, based on testing a combination of antioxidant extract of orange peel and leaf tea, then tested the combination of orange peel and tea leaves in order to provide an anticancer effect was the same even better than drugs for cancer, it can be seen from the IC50 value is 83, 03.Sehingga side effects of chemotherapy drugs can be lowered in conjunction with the decrease in dose.

4. Cytotoxic test with MTT Assay Method

Table 4. IC50 value Cytotoxic

Concentration	1	2	3	Average
31.25	.7787	.9036	.9391	0.91299
62.5	1,022	.9238	.924	.9566
125	1.0563	1.1727	1.0871	1.10536
250	1.0391	1.1489	1,186	1.1246
500	.9142	1.1093	1.1141	1.04586
1000	1.0559	.8894	1,068	1.00443
2000	1.0365	.8771	.8429	0.91883
IC50	**7707.576**			



From the results of the cytotoxic test data above indicate where the results are not good, this can occur for several reasons including the tools used are not sterile, causing contamination and cross-contamination can also occur. Then the test will be repeated to obtain good results.

5. Tablet Formulation With Wet Granulation Method

Table 5. Formulations Preparations Tabet

Materials	Formula 1	Formula 2
Tea leaf extract and orange peel	50 mg	50 mg
PVP	1%	3%
Manihot starch	5%	5%
Mg stearate	1%	1%
Talk	1%	1%
Lactose	qs	qs

Formula is made for 100 tablets weighing 270 mg tablet 0ER.
Formula 1: Concentration of PVP 1%
Formula 2: Concentration of PVP 3%

- Test weight uniformity

The average weight of 20 tablets = 14.436: 20 = 0, 7218

Table 6. Weight 20 Tablet

20 WEIGHT TABLET	
1 = 0.6867	
2 = 0.7216	11 = 0.7155
3 = 0.7058	12 = 0.7150
4 = 0.7204	13 = 0.7055
5 = 0.6952	14 = 0.7102
6 = 0.7095	15 = 0.7047
7 = 0.7211	16 = 0.7118
8 = 0.6942	17 = 0.7096
9 = 0.7029	18 = 0.7092
10 = 0.7023	19 = 0.6781
	20 = 0.6965

Of a weight of 20 tablets 1 tablet above are not passing average number of 20 tablets, so the average weight of the tablet which can be nice.

- Test compression / compressibility (<20%)

Requirements: no more than 20%.

Table 7. Test Results pemapatan

	Before	**After**	**result**
tubes A	126 ml	119 ml	5.55%
tube B	126 ml	114 ml	9.52%

Compression of the test tube A and tube B results in a great get for by the terms of the test itself tidaklebih compression of 20%

- Tablet hardness

  The average hardness of 10 tablets namely: 4.4979 kg, whereas a good tablet hardness is 4-9 kg.

  Table 8. Average hardness 10 Tablets

	WEIGHT (kg)
1	3.430
2	4,140
3	4,565
4	3,110
5	3,699
6	3,000
7	4, 255
8	6.670
9	5.630
10	6,480

CONCLUSION

The combination of ethanolic extract of orange peel and tea leaves have IC50 83.03ug / mlmennjukkan ethanolic extract and leaf tea has high antioxidants. Can also be seen from the results of molecular docking on the compound kaempferol -6.7 and -6.4 at -4.5 and -5.8 tangeretin compound, TLC results also indicate the presence of flavonoids in orange peel mandarin and tea leaves are the result of TLC 0.87 and 0.65. Based on this result, the combination of orange peel extract and tea leaves potential to be developed the finished product (in the form of standardized herbal supplements or drugs) are equipped with a variety of relevant supporting test safety. In this study has begun prototyping the combination product of orange peel extract and the tea leaves in the form of a tablet with uniformity of weight test result is 0.7218, the compression test on the tube A and B result in a nice get pemamatapn where the terms of the test itself is not more than 20%, the tablet hardness test results in get pretty good too. In the cytotoxic test results that get less good, less good results can be caused by various things are like tools used are not sterile, the materials used is contaminated, then re-tested AKN.

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The Antioxidant Activity Test on Combination of Ethanol Extract of Soursop (*Annona muricata L.*) and Tea (*Camellia sinensis*) Leaves Using DPPH (*1,1-diphenil-2- pikrilhidrazil*) Method

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Abstract

Soursop *(Annona muricata L.)* and tea *(Camellia sinensis)* leaves contains flavonoid compounds that have a strong antioxidant effect. Antioxidant activity is the effect of a compound which can prevent the oxidation reaction caused by the presence of free radicals. The purpose of this study is to know the antioxidant activity from the combination of ethanol extract of soursop and tea leaves. The antioxidant activity test was performed using DPPH method (*1,1-diphenil-2-pikrilhidrazil*). The soursop and tea leaves were macerated by using ethanol 70%. Identification of flavonoid compounds on a combination of ethanol extract of soursop and tea leaves was done by using TLC method with Silica Gel F_{254nm} as a stationary phase and *n*-Butanol : Acetic Acid : Water (BAW) (7:2:1) as mobile phase. Combination of ethanol extract of soursop and tea leaves were examined its antioxidant activity by a ratio of 1:1, which were made in several of concentration series and used vitamin C as a comparison. The antioxidant activity test by the DPPH method that was done by Vis Spectrophotometer with λ max 514 nm, until IC_{50} was obtained. The results of flavonoid identification by TLC method resulted in 2 spots with Rf first spots 0.66 and Rf of the second spots 0.89 which was the flavonoid group. Antioxidant activity obtains the IC_{50} value of 26.90 µg/mL and standard of vitamin C with IC_{50} equal to 14.20 µg/mL. The study showed that ethanol extract of soursop and tea leaves contained flavonoid compounds, and have very strong antioxidant activity with IC_{50} values approaching Vitamin C.

Keywords : Annona muricata L, Camellia sinensis, Antioxidant, DPPH, TLC

INTRODUCTION

There may be some theoretical background stated in the introduction. The causes of a disease such as cancer can vary, including the presence of free radicals in the body, where free radicals are highly reactive compounds (Juniarti *et al*., 2011). Natural free radicals are produced from the rest of the body's metabolism while free radicals from outside the body can be sourced from smoke, UV light and others. One of the effort to prevent the harmful effects of free radicals is to use antioxidant compounds. Biological antioxidants are compounds that can counteract or reduce the harmful effects of free radicals. Sources of anti-oxidant compounds can come from natural or synthetic materials (Sayuti, 2015).

Tea leaves (*Camellia sinensis*) have many benefits, the biggest of which is as a powerful antioxidant because inside the tea leaves (*Camellia sinensis*) there were some contents of active compounds such as polyphenols, compounds classified as polyphenols in tea, namely *catechins, epicatechin (EC), epicatechin- 3-gallate (ECG) and epigallocatechin-3-gallate (EGCG). Catechins* are powerful antioxidants and these compounds are thought to suppress cell proliferation and have chemo preventive effects (Towaha and Balitri, 2013). Besides tea leaves, soursop leaves (*Annona muricata L.*) contain compounds such as essential oils, *flavonoids, reticuline, coclaurine, higenamine, annomurine and acetogenin* (Yi *et al*., 2007). Flavonoid compounds contained in both plants are thought to have a function to counteract free radicals. This study aims to determine the presence of flavonoid compounds in a combination of tea leaf and soursop leaves extract and test the antioxidant activity of the combination of the two extracts.

MATERIALS AND METHOD

Materials

Tea leaves (*Camellia sinensis*) simplicia powder obtained from Wonosobo and soursop leaves (*Annona muricata L.*) from Bantul regency.

Sample Preparation

Powder samples of each leaf were weighed 500 g and extracted by maceration method using 70% ethanol solvent for 4x24 hours and remacerated for 2x24 hours. The filtrate obtained is then evaporated using a vacuum rotary evaporator with a temperature of 40-50^{o}C until a thick extract is obtained.

Flavonoid substances Identification by TLC Method

To identify flavonoids or polyphenols in the extract using the mobile phase, namely n-butanol: acetic acid: water (BAA) with a ratio of 7:2:1 while the stationary phase used is silica gel GF_{254nm}. After elution of the TLC plate was removed from the chamber and dried in an oven at 600^{o}C for 10 minutes, then the plate was observed under UV light with a

wavelength of 254 nm and the results and Rf were analyzed. After that, the color reagent is used which is ammonia reagent to detect flavonoids.

Antioxidant Activity Test

1. **Preparation of DPPH Solution**
   A total of 15.8 mg of DPPH powder was put into a measuring flask, then dissolved in 100 mL of methanol p.a to obtain a concentration of 0.4 mM. The solution was mixed by a vortex for 30 seconds then wrapped using aluminum foil.

2. **Preparation of Vitamin C Solution**
   Total of 5 mg of vitamin C was put into a 50 mL measuring flask, then methanol p.a was added to the boundary markers as the mother liquor. Then made a series of 0.5 vitamin C levels; 1; 5; 10; 20; 30 µg / mL.

3. **Preparation of Sample Solution**
   A total of 20 mg combination of ethanolic extract of soursop leaves and tea leaves was added with 20 mL of methanol p.a to obtain a concentration of 1000 µg/mL. From the mother liquor, a level 5 series is made which were 7.5; 10; 15; 20; 30 µg/mL.

4. **Determination of Operating Time**
   Prepared of 3 measuring flasks 5 ml, then pour vitamin C solution of 3; 4; 5 µg/mL as much as 1 mL to three volumetric flask in sequence. Then added of 1 mL of DPPH solution into each measuring flask and add methanol to the boundary markers. Transfer each solution in a measuring flask to a test tube then 30 second Vortex. Read the absorption of the solution at λ 514 nm every 5 minutes for 45 minutes, replication 3 times.

5. **Determination of Maximum Wavelength**
   In a 10 mL volumetric flask put 1 mL of DPPH solution, then added by methanol to the boundary mark. The solution was transferred to a test tube. Mixed the solution for 30 seconds. Let stand for operating time to read absorption at a wavelength of 200-800 nm.

6. **Measurement of Absorbance of DPPH Solution**
   In a 10 mL volumetric flask, 2 mL of DPPH solution was added. Then added methanol to the boundary mark then let stand for operating time. Read uptake at λ maximum, replicated by 3 times.

7. **Measurement of Absorbance of Vitamin C and Samples Solution**
   In a 10 mL volumetric flask, 2 mL of DPPH solution was added. Added by 2 mL of vitamin C solution or sample solution to various concentrations. Then added by methanol to the boundary markers. Mixed the solution for 30 seconds, and let stand for operating time. Read uptake at λ Maximum and replicate 3 times.

To calculate IC_{50} by processing the sample absorbance data into a form of % antioxidants with the following formula:

$$\% \text{ inhibition} = \frac{\text{blanko absorbantion-sample absorbantion}}{\text{blanko absorbantion}} \times 100\%$$

IC_{50} values can be obtained by entering a value of 50 as y in the linear regression equation obtained from the relationship x = levels and y =% antioxidants.

RESULT AND DISCUSSION

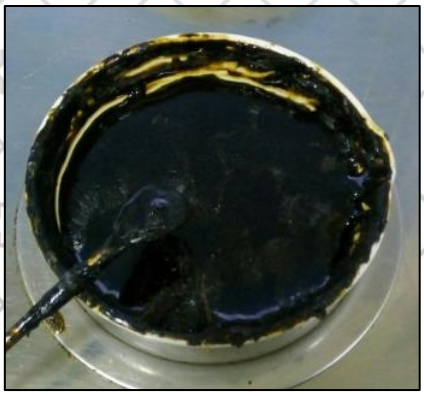

Figure 1. The result of extraction process

The results of maceration then evaporated by rotary evaporator and waterbath obtained a thick extract of *Camellia sinensis* leaves weighing 27.3 grams and the leaves of *Annona muricata* L. weighing of 28.7 grams. The ethanol extracts of tea leaves and soursop leaves had produced yields of 13.38% and 14.6%, respectively. The yield of soursop leaf ethanol extract has a higher value than the extract or the tea although the difference is not too much. Extract yield was calculated based on the ratio of final weight (weight of extract produced) to initial weight (sample weight used) multiplied by 100%.

Identification of Flavonoid Substances by TLC Method

From this experiment the BAW mobile phase with a ratio of 7:2:1 showed the best result of the separation compared to other comparisons.

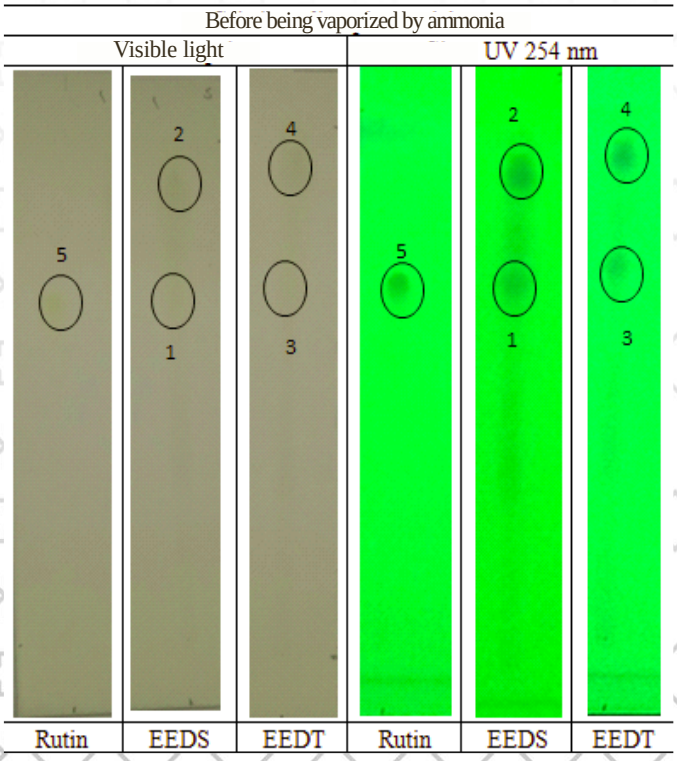

Figure 2. Sample before being vaporized by ammonia

To detect the presence of flavonoids, color testing was carried out. The method that used was evaporation with ammonia compounds, routine chromatogram plates, EEDT and EEDS with ammonia for a few moments until color changes occur in the spots that appeared.

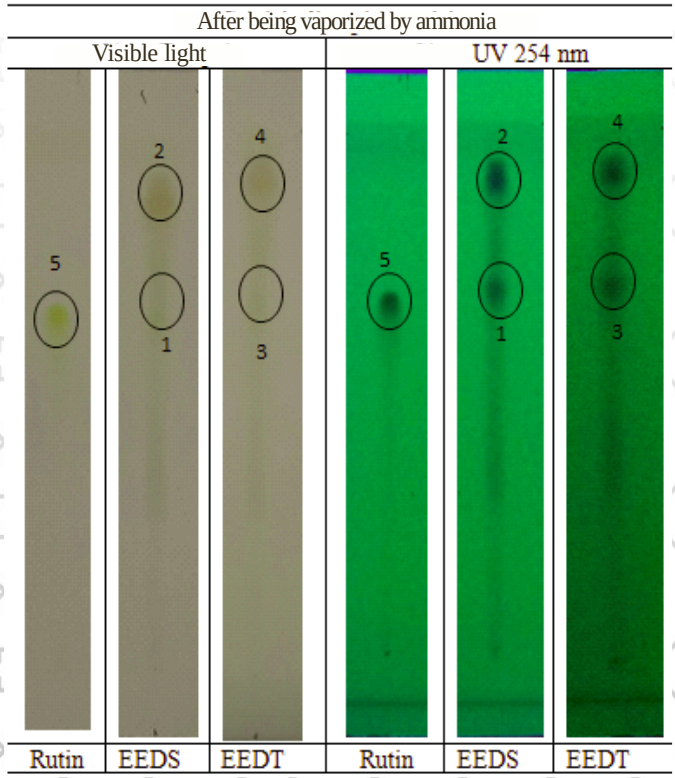

Figure 3. Sample after being vaporized by ammonia

Detection using visible light and UV light 254 nm produced 2 spots on the EEDT chromatogram and 2 spots on the EEDS chromatogram which were compared to the routine standard. The Rf value of each speck can be seen in the table 1.

Table 1. Rf value and spotting colour

Spotting number	Rf	Spotting colour before being vaporated		Spotting colour after being vaporated	
		Visible	UV 254 nm	Visible	UV 254 nm
1	0,66	Colorless	Damping	Yellow	Darker damping
2	0,89	Colorless	Damping	Violet Yellow	Darker damping
3	0,68	Colorless	Damping	Yellow	Darker damping
4	0,91	Colorless	Damping	Violet Yellow	Darker damping
5	0,66	Colorless	Damping	Yellow	Darker damping

EEDT and EEDS containing flavonoids in both spots were proven by yellow changes after ammonia and blue (damping) in UV 254 nm. Based on the results of identification using TLC, EEDT samples with number 1 and 2 spots that had Rf of 0.66 and 0.89 and EEDS samples with spots number 3 and 4 which Rf value of 0.68 and 0.91, respectively, thought to contain *flavonoid* compounds. The four patches were darkened in blue at UV 254 nm and colorless in visible light before being evaporated with ammonia. After being evaporated with ammonia on the rays, it can be seen that the spots are dim yellow which indicates the flavonoid content (Aminah and Pramono, 2013) in the form of flavonoid glycosides in spots 1 and 3, in accordance with the comparable Rf value used which is the routine showed in spot number 5 with the Rf value 0.66. This has consistent result with previous studies that routine Rf values ranged between 0.625 and 0.75 by BAW mobile phase, the possibility of routine compound in the extract was quercetin (Suhendi, 2011). Quercetin is a flavonoid derivative classified as flavonol, as well as catechins found in tea leaves and soursop leaves which are also included in flavonol (Neldawati, 2013). So that routine use as a comparison is suitable to detect flavonoid content. In spots number 2 and 4 is not a flavonoid glycoside compound because it has a different and higher Rf, it was suspected that this flavonoid compound was genistein type because genistein is a less polar flavonoid (Andersen and Markham, 2006). The Rf values of the two samples were almost similar, this indicated that the flavonoid compounds in the EEDT and EEDS samples have similar types of compounds.

Antioxidant Activity Test

From the results of the experiment, a series of levels obtained for Vitamin C were 0.5; 1; 2; 5; 10; 20 and 30 µg/mL, while the concentration series for the samples were 5; 7.5; 10; 15; 20 and 30 µg/mL. Each sample were replicated in 3 times. Absorption readings were carried out at the maximum wavelength of DPPH solution, from the results of screening

the maximum wavelength of DPPH using a spectrophotometer, the maximum wavelength obtained was 514 nm. The average data of absorbance reaction between DPPH with Vitamin C and DPPH with a combination of EEDT and EEDS can be seen in the table 2.

Table 2. Average Absorbance of vitamin C

No	Concentration (µg/mL)	Average absorbance	Standard Deviation
1	0,5	0,641	0,026
2	1	0,638	0,006
3	2	0,625	0,034
4	5	0,557	0,013
5	10	0,402	0,021
6	20	0,189	0,063
7	30	0,055	0,014

Table 3. Average Absorbance of EEDT and EEDS sample

No	Concentration (µg/mL)	Average absorbance	Standard Deviation
1	5	0,661	0,016
2	7,5	0,598	0,022
3	10	0,532	0,003
4	15	0,488	0,017
5	20	0,476	0,061
6	30	0,298	0,018
7	40	0, 186	0,022

From the average absorbance can be used to calculate the percentage of inhibitory concentration (% IC). The percentage of inhibitory concentration was obtained by the absorbance of the negative control reduced by the absorbance of the positive control then the results were divided by the absorbance of the negative control. To get the absorbance of the negative control, the absorbance of the DPPH solution was read. From the results of the experiment, the average absorbance of DPPH was 0.7023. Data from the calculation of the percentage of inhibition of Vitamin C can be seen in the table 4.

Table 4. Data of Inhibition percentage of Vitamin C

Concentration µg/mL	Absorbance	Blanco Absorbance	Percentage of Inhibition (%)
0,5	0,641	0,7023	8,73
1	0,638	0,7023	9,11
2	0,625	0,7023	11,01
5	0,557	0,7023	20,76
10	0,402	0,7023	42,76
20	0,189	0,7023	73,04
30	0,055	0,7023	92,22

Table 5. Data of Inhibition percentage of EEDT and EEDS sample

Concentration µg/mL	Absorbance	Blanco Absorbance	Percentage of Inhibition (%)
5	0,661	0,7023	5,88
7,5	0,598	0,7023	14,90
10	0,532	0,7023	24,30
15	0,488	0,7023	30,56
20	0,476	0,7023	32,27
30	0,298	0,7023	57,53
40	0,186	0,7023	73,54

To determine the antioxidant effect of the combination of EEDT and EEDS and Vitamin C, the IC_{50} values were calculated by making the relationship between percent inhibition and concentration then linear regression. Linear regression graph of the relationship between concentration and percent inhibition of the combination of EEDT and EEDS along with Vitamin C can be seen in the following graph.

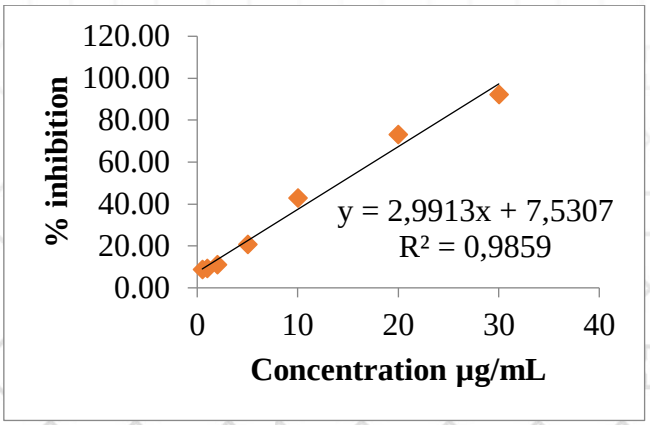

Figure 4. Graphic of Linear Regression Vitamin C

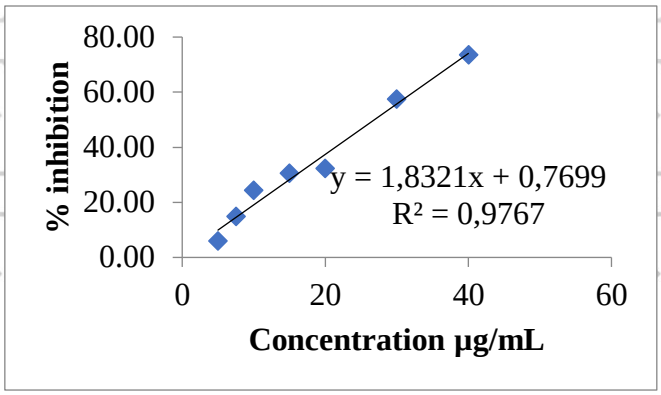

Figure 5. Graphic of Linear Regression Vitamin C

After obtaining a linear regression equation, IC50 can be calculated. This value was obtained by changing the value of y to 50. The calculation results can be seen in table 6.

Table 6. Value of IC $_{50}$

Sample	Value IC_{50} (µg/mL)	Notes (Mardawati *et al*., 2008)
Vitamin C	14,20	Very strong
Combination of EEDT and EEDS	26,90	Very strong

From the linear regression equation, IC_{50} values can be calculated, the combination of ethanol extract of *Camellia sinensis* leaves and *Annona muricata L.* leaves has an IC_{50} value of 26.90 µg/mL. According to Mardawati (2008), the results of the antioxidant activity test from a combination of ethanol extract of *Camellia sinensis* leaves and *Annona muricata L.* leaves showed that the activity was categorized as very strong as an antioxidant. The IC_{50} value obtained from the test solution was greater than the comparison

solution of vitamin C which has an IC_{50} value of 14.20 µg / mL. This result showed that vitamin C as a positive control has a higher antioxidant activity when compared with a combination of ethanol extract of *Camellia sinensis* leaves and *Annona muricata L.* leaves but the extract had a very strong effect as antioxidant.

CONCLUSION

In a combination of ethanolic extract of *Camellia sinensis* leaves and *Annona muricata* L. was proved that the extract containing flavonoid group compounds, as evidenced by the Rf EEDS values of 0.66 and 0.89. While EEDT with Rf was 0.68 and 0.91. Besides that, it was proved by the color reagent that ammonia vapor gave yellow to violet yellow.

While the antioxidant activity of a combination of ethanolic extract of *Camellia sinensis* and *Annona muricata* L. leaves based on DPPH method has an IC_{50} value of 26.90 µg/mL which has very strong activity.

ACKNOWLEDGEMENT

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ICPU_2019_005

The Comparison of Nobiletin Compound With 5-Fluorouracil As Breast Cancer Cemopreventive Agents According To *In-Silico*

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Abstract

The incidence of breast cancer is still increasing. Currently, the therapy strategy is limited to chemotherapy, radiation, and surgery. In addition to the expensive drugs, the side effect is still fraught with several challenges. The used chemotherapy drug, for example, 5-Fluorouracil which has a side effect to kill the normal cell. Indonesia has abundant nature potential which has the potential to be developed into drugs. One of them is Bandotan Herbs (Ageratum conyzoides L.) which proved has chemopreventive potency. It's nobiletin compound allegedly potential to be chemopreventive agents in breast cancer.

Objective:This study aims to investigate the potential binding between nobiletin with VEGF and COX as protein targets compared to a chemotherapy drug, 5-FU.

Method:The method used computational analysis with molecular docking principle with Autodock Vina.

***Results*:** The results showed that nobiletin could be used as a chemopreventive agent, the RMSD values obtained for nobiletin compounds were 1,122 and 1,038 while for 5-FU compounds were 1,534 and 1,927. The docking score obtained is -7.6 kcal/ mol (nobiletin to VEGF), -7.5 kcal/ mol (nobiletin against COX-2), -4.7 kcal/ mol (5-FU to VEGF) and -5.2 kcal/ mol (5-FU against COX-2).

Conclusion: *It* can be concluded that nobiletin potential to be developed into chemopreventive agents for breast cancer.

Keywords : Bandotan, 5-FU, VEGF, COX-2, molecular docking

INTRODUCTION

Cancer described by uncontrolled cell division. This is caused by DNA damage resulting in mutations of vital genes that control cell division. Cell differentiation and cell growth regulated by protooncogen and tumor suppressor genes found in all chromosomes in large numbers. Protooncogens that have undergone changes that give rise to cancer called as oncogenes (Kumar, 2005). Neoplasm or cancer is a cell growth that occurs in the body, shape and characteristic both are different from normal cell. The damaging of it's shape and function due to cells that grow apart from the normal cell control system. Neoplasm occurs because there is a transformation of normal cells due to the genes flaw that regulate cell differentiation (Sukardja, 2000).

Breast cancer remains a major public health problem. The incidence is rising in most countries and is projected to rise further over the next 20 years (Rahib L., et al 2014). In most countries, an increase in numbers of women with major breast cancer risk factors, including lower age of menarche, late age of first pregnancy, fewer pregnancies, shorter or no periods of breastfeeding, and a later menopause (Colditz GA, Bohlke K, 2014). Breast cancer spreads through the lymph system by entering the vessel and growing in the gland. Abscess of the lymph nodes in the armpit occur if breast cancer cells enters to the bloodstream and reached the lymph vessel in the armpits, this indicates that most likely cancer cells have spread to other organs (Soebachman, 2011).

Indonesia has total population 247,000,000 people. The highest cancer incidence was achieved by breast cancer with 48,998 cases obtained from WHO in 2014. Death caused by breast cancer shows 21,4% from 92,200 cases in Indonesia. According to the latest data, WHO 2017 the death cases caused by breast cancer reached 21,287 people.

Nowadays, there are three ways to treat cancer using chemotherapy, radiation and surgery. One therapy that is often used for cancer treatment is chemotherapy. Chemotherapy agents has a narrow safety limitation. It works not selective by damaging both normal cell and cancer cells (Dai, et al., 2004).

Therefore, it is important to find the integrated chemotherapy or chemopreventive agents which is by using alternative medical herbs to be developed as western medicine with natural based. The previous research compared the potential of nobiletin due to 5-FU in Bcl-xl protein, we got the docking score -4.7 kcal/mol for 5-FU and -8.0 kcal/mol for nobiletin. It means that nobiletin can be used as chemopreventive agents due to breast cancer.This paper will discuss about the chemopreventive activity of nobiletin and 5-Fluorouracil for breast cancer based on in silico study (Komalasari, 2017).

MATERIALS AND METHOD

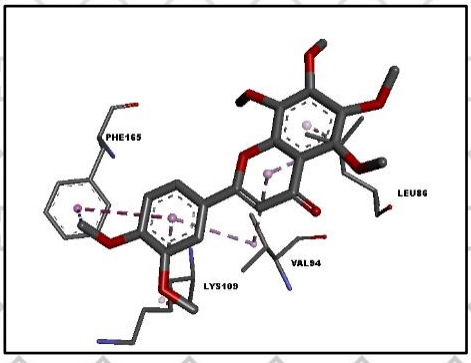
Autodock Vina an aplication for molecular docking. It was obtained for free by visiting http://vina.scripps.edu/download.html. Autodock Vina application was run with MGL

Tools, Phyton and Open Babel. DS Visualizer used to prepare proteins target and ligands. We used RMSD calculation and binding activities.

The structure of protein target was taken from the Protein Data Bank (PDB) by clicking www.rcsb.org with PDB ID 51KQ for COX-2 protein, while VEGF is using 5XV7.

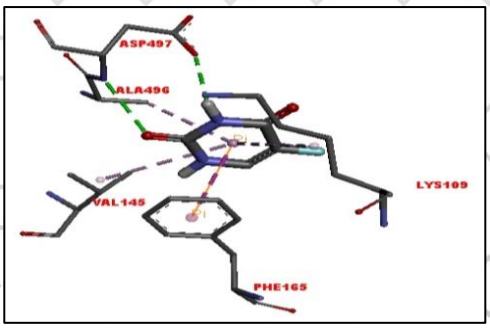
RESULT AND DISCUSSION

a. Visualization of Nobiletin to VEGF



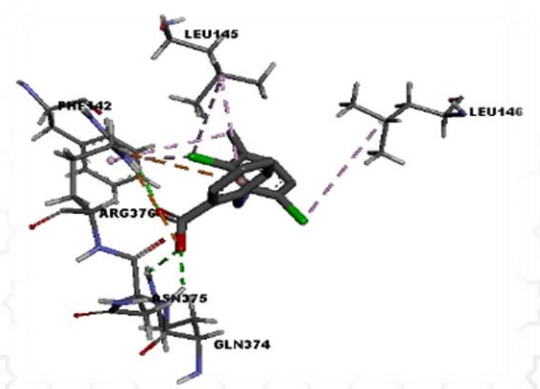
Docking score : -7,6 kcal/mol

b. Visualization of 5-FU to VEGF



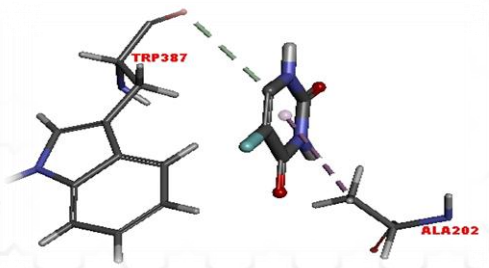
Docking score : -4,7 kcal/mol

c. Visualization of Nobiletin to COX-2



Docking score : -7,5 kcal/mol

d. Visualization of 5-FU to COX-2



Docking score : -5,2 kcal/mol

Compound	Protein	RMSD	Docking Score	Conformation
Native Ligand	VEGF	1.935	-10.7	2
	COX-2	1.578	-7.4	6
Nobiletin	VEGF	1.122	-7.6	6
	COX-2	1.038	-7.5	4
5 - FU	VEGF	1.534	-4.7	3
	COX-2	1.927	-5.2	6

Each compound will produce 9 conformations with different RMSD values so that conformation with RMSD value <2Å is chosen. RMSD is the deviation value between a ligand conformation and its comparison, that is, if the deviation is too large, the greater the prediction error of ligand and protein interactions (Korb, et al., 2006). The Nobiletin compound was chosen the 6th conformation with the RMSD value of 1.122 Å and the docking score of -7.6 kcal / mol while for VEGF the conformation was the 4th with RMSD 1.038 and scor docking -7.5 kcal / mol. Native ligand has a docking score of -10.7 and 7.4 kcal / mol respectively, higher than Nobiletin. 5-FU has a docking score of -4.7 kcal / mol andd -5.2 kcal / mol, higher than Nobiletin and original ligand so that the interaction stability of Nobiletin is better than 5-FU and the original ligand for VEGF and COX-2 proteins .

Based on previous research, nobiletin has been shown to have antitumor activity through antiproliferation mechanisms, induction of apoptosis, cell cycle deregulation (Yoshimizu, et al., 2004), antiangiogenesis (Wang, et al., 2014), anti-inflammatory and anticarcinogenic (Li, et al. 2008) and other pharmacological activities through in vitro and in vivo tests (Huang, et al., 2016). As for the results of this study, Nobiletin has potential as anticancer activity through an apoptotic mechanism involving VEGF and COX-2 proteins by in silico study.

CONCLUSION

Nobiletin compound has potential to be developed as chemopreventive agents in breast cancer.

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ICPU_2019_006

Potential Combination Of Ethanolic Extract Tea Leaves (*Camelia sinensis. L.*) and Soursop Leaves (*Annona muricata. L.*) as a Chemopreventive Agent for Colon Cancer by Antioxidant Test and Molecular Docking

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Abstract

Cancer can be characterized by uncontrollable cell division. Colon cancer is the biggest third number in the world that can causing a death. For cancer treatment, we have to use a special drug and usually that drug has narrow theraphy index. So, it can causes some side effects. So, with developing the agent of chemopreventive that derived from natural materials, hopefully can be an effective anticancer drug with the smaller side effects. Tea leaves (*Camelia sinensis L.)* and soursop leaves (*Annona muricata L.)* contain a flavonoid that can have an antioxidant activity, so it can prevent a cancer cell. The purpose of this research are want to know the anticancer activity based on the combination between tea leaves and soursop leaves.

Tea leaves and soursop leaves already extracted with maceration and also evaporated to get a viscous extract. For identify the content of those leaves, we use Thin Layer Chromatography (TLC) and for knowing the activity of antioxidant, we use DPPH method with vitamin C as a comparison. Then, for in silico test we use molecular docking with prepare an EGCG and Acetogenin, so they can use bcl-xl as a target protein.

Based on 500 gram tea leaves, we get a 27,3 gram viscous extract and also from 500 gram soursop leaves, we get a 28,7 gram viscous extract. The Rf result from TLC is 0.77 gram for tea leaves and 0.98 gram from soursop leaves. The antioxidant activity combination EEDTDS show us that the IC_{50} is 26.90 µg/mL. For the docking molecular , it show that the activity of EGCG and Acetogenin can inhibit bcl-xl target protein with docking score -8.1 and -6.7 kkal/mol. From those result, can be concluded that EEDTDS have a chemopreventive potention for colon cancer.

Keywords : Annona muricata L, Camellia sinensis, Colon Cancer.

INTRODUCTION

Cancer can be characterized by uncontrollable cell division. According to WHO (2005) declare that 7.6 million people died due to cancer. Colon cancer is the biggest third number in the world that can causing a death. The higest causes of colon cancer is about a bad lifestyle such as consuming alcohol, fastfood, smoking and also exposure to free radicals. Beaucse most of the diseases caused by free radicals. Free radicals have a very reactive and very fast half-life. Free radicals will react quickly by taking electrons surrounding molecules, it can lead to excessive oxidation reactions in the body and causing a damage to normal tissue.

In addition, a large amounts of free radicals can causing interference with the production of DNA, lipid layer on the walls of the cells and reduces the ability of the cell to adapt to its environment. Oxidative stress induced by free radicals has been note can influence the occurrence of degenerative diseases such as cancer, coronary heart disease and premature aging. As for sources of free radicals are divided into two endogenous and exogenous. Endogenous free radicals derived from autoxidation where free radicals produced from aerobic metabolic processes of the body that is taking place. While exogenous free radicals derived from radiation, drugs, smoke cigarettes and the vehicle (Kikuzaki et al., 2002). The body requires adequate amounts of antioxidants in to temper the impact of free radicals. Antioxidants can be sourced from the diet and intake of other nutrients.

Tea leaves (*Camelia sinensis L.*) has many benefits including as anticancer, antioxidant, antibacterial and others (Alamsyah, 2006). Tea leaves contain active compounds including EGCG, caffeine, theobromine, the oil of life (Fulder, 2004). Epigallocatechin gallate (EGCG) is the main polyphenols found in tea leaves which is effective as an antioxidant and a bitter taste. In while, soursop leaves (*Annona muricata L.*) contain compounds including tannins, phytosterols, calcium oxalate, alkaloids, flavonoids, and acetogenin (Wulan, 2012). The purpose of this research are want to know the anticancer activity based on the combination between tea leaves and soursop leaves.

MATERIALS AND METHOD

Materials and Tools

Simplicia of tea leaves (*Camelia sinensis L.*) and soursop leaves (*Annona muricata L.*), DPPH, ethanol 70%, vitamin C, methanol, tools glass (pyrex), glass, quartz timepiece kuvet, shelves, micro test tubes pipette (Gilson), aluminum foil, a horn spoon drops, eyedropper, bowls porcelain, analytic scales (Sartorius), UV-Vis spectrophotometer (Shimadzu), Asus computer hardware, molecular docking software Autodock Vina.

Sample Preparation

From each of simplisia were weighed as much as 500 grams by maceration method using 70% solvent ethanol and remaserase for 2x24 hours. The filtrate obtained is then

evaporated using a vacuum rotary evaporator with a temperature of 40-50 ^{0}C until a thick extract is obtained.

Identify Flavonoid substances using TLC Method

To identify flavonoids or polyphenols in the extract using the mobile phase, namely acetic etil: glacial acetic acid: formic acid: aquadest with a ratio of 100:11:11:27 (v/v) (Brraseur dan Angenot, 1986) while the stationary phase using silica gel GF254. After elution of the TLC plate was removed from the chamber and dried in an oven at 600 C for 10 minutes, then the plate was observed under UV light with a wavelength of 254 nm and the results and Rf were seen. After that, the color reagent is used which is ammonia vapor to detect flavonoids.

Antioksidant Activity Test

1. **Preparation of DPPH raw Solution**
   A number of 15.8 mg powder DPPH was put into the pumpkin, then measure out in 100 mL of methanol p.a to obtain a contentration of 0.4 mM. Solution in vortex for 30 seconds then wrap using aluminum foil.

2. **Preparation of Aqueous Samples**
   Samples are removed from freezer and keep in a normal temperature. A total of 20 mg combination of ethanolic extract tea leaves and soursop leaves was added with 20 mL methanol p.a to obtain a concentration of 1000 µg / mL. From the mother liquor, a level 5 series is made; 7.5; 10; 15; 20; 30 µg / mL.

3. **Making Vitamin C Solution**
   A total of 5 mg of vitamin C analysis was put into a 50 mL measuring flask, then methanol p.a was added to the boundary markers as the mother liquor. Then made a series of 0.5 vitamin C levels; 1; 5; 10; 20; 30 µg / mL.

4. **Determining Operating Time**
   Prepare 3 measuring flasks 5 ml, then pour vitamin C solution 3; 4; 5 µg / mL as much as 1 mL to 1, 2, 3 volumetric flask in sequence. Add 1 mL of DPPH solution into each measuring flask and add methanol to the boundary markers. Transfer each solution in a measuring flask to a test tube then 30 second Vortex. Read the absorption of the solution at λ 514 nm every 5 minutes for 45 minutes, replication 3 times.
5. **Maximum Wavelength Assignment**
   In a 10 mL volumetric flask put 1 mL of DPPH solution, then add methanol to the boundary mark. Then move the solution into test tubes, vortex solution for 30 seconds. Let stand for operating time, and then see the absorption at a wavelength of 200-800 nm.

6. **Measurement of Absorbance DPPH Solution**
   In a 10 mL volumetric flask, 2 mL of DPPH solution were added. Add methanol to the boundary mark then let stand for operating time. Read uptake at λ Maximum, replicate 3 times.

7. **Measurement of the absorbance of the sample solution and vitamin C**
   In a 10 mL volumetric flask, 2 mL of DPPH solution were added. Add 2 mL of vitamin C solution or sample solution to various concentrations. Then add methanol to the boundary markers. Vortex solution for 30 seconds, and let stand for operating time. Read uptake at λ Maximum and replicate 3 times.

   To calculate IC_{50} by processing the sample absorbance data into a form of % antioxidants with the following formula:

$$\% \text{ inhibition} = \frac{\text{blanko absorbantion} - \text{sample absorbantion}}{\text{blanko absorbantion}} \times 100\%$$

IC_{50} values can be obtained by entering a value of 50 as y in the linear regression equation obtained from the relationship x = levels and y =% antioxidants.

Molecular Docking Test

1. **Protein Preparation**
   We get the target protein from PDB (Protein Data Bank) and bcl-xl from GDP. 1 YSG that already used as the target in the form of active protein will be bind into the native ligand. Then, the native ligand will be removed by YASARA process aims to provide the space. This space is used to analize the interaction between ligand and protein.

2. **EGCG and Acetogenin Compund Test Preparation**
   The optimization of the structure test compound is carried out using Marvin Sketch program. This 3D structur of EGCG and Acetogenin are full drawn with a hydrogen atom, and the conformation.

3. **Molecular Docking Validation**
   Native ligand already removed from protein using PLANTS program. The difference in the coordinates between two ligans can be observe from RSMD value. If the RSMD value is <2.0 Å, the protocol is accepted and the docking process of the compound test can be carried out. But if the RSMD value >2.0 Å, then we have to use another protein code.

4. **Docking of EGCG and Acetogenin on Target Protein**
   The test compound of EGCG and Acetogenin already eliminated native ligand using its program PLANTS. The results of the analysis will show the compounds with the conformation in the lowest energy that has to bind to a protein target and the

visualization of the amino acid residues that interact with the ligand can using PLANTS program.

Cytotoxic Test

1. **Reagen Preparation**
   To make MTT stock solution, dissolve 500 mg MTT poweder into 10 ML phosphate buffer solution. Then, strir the solution with a magnetic stirrer about 1 hour. Filter the sterilized solution with a 0.22 mm filter and then store it into 10 mL aliquots (50 mg/mL) at -20°C (van Meerloo et al., 2011). The result of the solution (5 mg/mL) will be prepared on the day of the research by dilution.

2. **Protocol of Cytotoxic Test**
   Cells were planted in a 96-well flat-bottom microtiter plate, so we can get $5x10^3$ cells/well and it has to incubate for 48 hours. After 48 hours, the culture medium should be replaced with a fresh medium. Subsequently, 100 µL of culture media and 10 µL of MTT added into each well, incubate again for 4-6 hours at 37°C. To stop the MTT reaction, use HCL 4N: Isopropanol (1:100), and shake it above shaker for 10 minutes. Finally, the intensity of the dissolved formazan crystals (purple) was quantified using ELISA reader at 595 nm.

RESULT AND DISCUSSION

The results of maceration using rotary evaporator and waterbath obtained a thick extract of *Camellia sinensis* leaves weighing 27.3 grams and the leaves of *Annona muricata L.* weighing 28.7 grams. Ethanol extracts of tea leaves and soursop leaves produced had yields of 13.38% and 14.6% respectively. The yield of soursop leaf ethanol extract has a higher value than the extract or tea although the difference is not too much.

Antioxidant Activity Test

Based on experiments that have been conducted the sample levels i.e. 5 series; 7.5; 10; 15; 20; 30 µg/mL. Each sample was replicated 3 times. While the series of levels for vitamin C that is 0.5; 1; 2; 5; 10; 20 µg/mL. each of the levels of vitamin C obtained replicated as much as 3 times. Absorbance reading of respective series samples and vitamin C levels is done at a wavelength of maximum DPPH. Based on the results of the screening DPPH maximum absorption readings using a spectrophotometer is 514 nm. The average data of absorbance reaction between DPPH with Vitamin C and DPPH with a combination of EEDT and EEDS can be seen in the following table.

Table 1. The average absorbance of the combination of ethanolic extracts of leaves of tea and soursop leaf

No	Concentration (µg/mL)	The Average Absorbance	Standard Deviation
1	5	0,661	0,016
2	7,5	0,598	0,022
3	10	0,532	0,003
4	15	0,488	0,017
5	20	0,476	0,061
6	30	0,298	0,018
7	40	0, 186	0,022

Table 2. The average absorbance of vitamin C

No	Concentration (µg/mL)	The Average Absorbance	Standard Deviation
1	0,5	0,641	0,026
2	1	0,638	0,006
3	2	0,625	0,034
4	5	0,557	0,013
5	10	0,402	0,021
6	20	0,189	0,063
7	30	0,055	0,014

From the absorbance data, can be used to calculate Percent Inhibition (% IC). The percentage of inhibition was obtained by the absorbance of the negative control. To get the absorbance of the negative control, the absorbance of the DPPH solution was read. From the results of the experiment, the average absorbance of DPPH was 0.7023. Data from the calculation of percent inhibition of Vitamin C can be seen in the following table.

Table 3. Percent inhibition of combination ethanolic extract of tea leaves and soursop leaves

Concentration µg/mL	Absorbance	Blanco Absorbance	Percent Inhibition (%)
5	0,661	0,7023	5,88
7,5	0,598	0,7023	14,90
10	0,532	0,7023	24,30
15	0,488	0,7023	30,56
20	0,476	0,7023	32,27
30	0,298	0,7023	57,53
40	0,186	0,7023	73,54

Table 4. Percent inhibition of vitamin C

Concentration µg/mL	Absorbance	Blanco Absorbance	Percent Inhibition (%)
0,5	0,641	0,7023	8,73
1	0,638	0,7023	9,11
2	0,625	0,7023	11,01
5	0,557	0,7023	20,76
10	0,402	0,7023	42,76
20	0,189	0,7023	73,04
30	0,055	0,7023	92,22

IC_{50} value is obtained by making the connection between the percent inhibition and concentration. Then, create a linear regression. The graph of a linear regression relationship between percent concentration with percent inhibition of vitamin C and the combination of ethanol extracts of leaves of tea and soursop leaf can be seen in the following table:

Figure 1. Graphic of Vitamin C Linear Regression

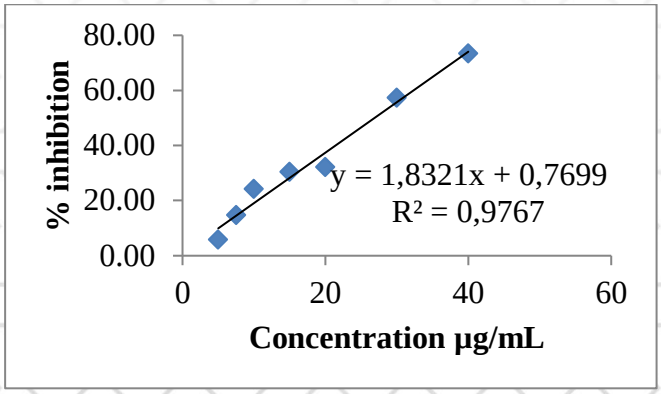
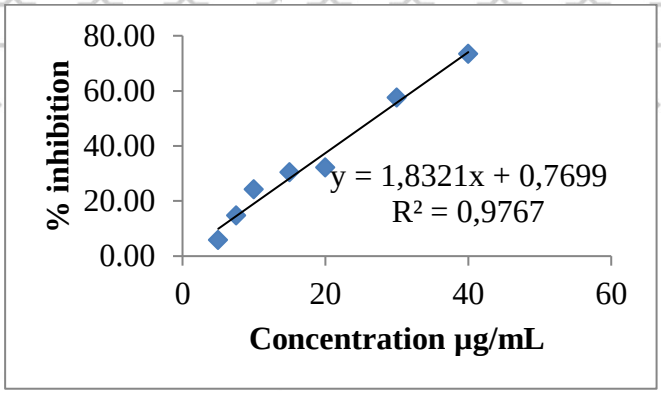


Figure 2. Graphic of linear regression combination of ethanol extracts tea leaves and soursop leaves



After obtaining a linear regression equation, IC50 can be calculated. This value is obtained by changing the value of y to 50. The calculation results can be seen in the following table.

Table 5. IC_{50} Value

Test compound	IC_{50} Value (µg/mL)	Notes (Mardawati *et al.*, 2008)
Vitamin C	14,20	Very Strong
Combination EEDT and EEDS	26,90	Very Strong

From the data, the IC_{50} values can be calculated. The combination of ethanol extract of tea leaves and soursop leaves has an IC_{50} value of 26.90 µg / mL. According to Mardawati in 2008, the results of the antioxidant activity test from a combination of ethanol extract of *Camellia sinensis* leaves and *Annona muricata L.* leaves showed that the activity was categorized as very strong as an antioxidant. The IC_{50} value obtained from the test solution is greater than the comparison solution of vitamin C which has an IC_{50} value of 14.20 µg / mL. It shows that vitamin C as a positive control has a higher antioxidant activity when compared with a combination of ethanol extract of tea leaves and soursop leaves.

Molecular Docking Trials

Molecular docking test aims to find the interaction of ligand with the protein molecules of the target before doing in-vitro test. Molecular docking can use Autodock Vina applications to visualize the structure and form of the interactions between ligand and protein molecular. The protein that tested in this study is bcl-xl. RSMD value and score of this test can be seen in the following table.

Table 6. Docking score of EGCG and Acetogenin against Protein bcl-xl

Compound name	Treatment	Protein	RMSD Value (<2.00 Å)	Docking score	3D Conformation	Amino Acid Conformation
Native Ligand			0.992	-6.4	Terlampir	Konformasi 2
EGCG		BCL-xl	0.284	-8.1	Terlampir	Konformasi 2
Acetogenin	Combination	(Code Protein: 1YSG)	1.567	-6.7	Terlampir	Konformasi 5
Doxorubicin			1.207	-3.6	Terlampir	Konformasi 2
5-FU			1.439	-4.9	Terlampir	Konformasi 2

The difference in the coordinates between two ligans can be observe from RSMD value. If the RSMD value is <2.0 Å, the protocol is accepted and the docking process of the compound test can be carried out. But if the RSMD value >2.0 Å, then we have to use another protein code. The result is, the docking score of EGCG is -8.1 kcal/mol, acetogenin is -6.7 kcal/mol. Those result compared with Doxorubicin and 5 FU with the docking score -3.6 kcal/mol and -4.9 kcal/mol. So, the EGCG and Acetogenin docking score are lower than doxorubicin and 5 FU. It means that the compound of EGCG and Acetogenin are stronger than those two.

Cytotoxic Test

In the present study, the systematic experimental steps in order to determine the potential cytotoxicity of drug at different concentrations by MTT assay are presented in video form. It is shown that a decreasing absorbance at 540 nm in the cells treated with increasing concentration of the drug in comparison to the control cells without any treatment. A decrease absorbance in the cells are treated with drug suggesting cytotoxicity. MTT assay significantly helps the researchers to determine whether any of the test compounds has cell toxicity or proliferative activity (Alley et al., 1988, Mosmann et al., 1983).

In this study, microtitter plate are used to attached the cells. That is why, flat-bottomed 96-well microtiter plate is preferred. But in case of suspension cells, either round bottom wells or flat bottom wells are used. The number of cells in the microplate is not unique for different cell lines and primary cells. The number of cells in the microtiter plate must be optimum to get good result. The number of cells is influenced the level of mitochondrial activity and the rate of proliferation. To get the optimum result, several concentrations of cells should be plated in 5-7 plates. Then, measure the optical density using colorimeter daily to determine the growth curve of the cell line to prevent overgrowth, which will influence the experiment. The starting optical density value of day 0 should not exceed 0.125 (van Meerloo et al., 2011). The optimal concentration of plating is established when cells have no lag phase. Then the assay should not proceed further after the log phase.

Table 7. Result of Cytotoxic activity combination of ethanolic extracts tea leaves and soursop leaves

Log Concentration	% Living of Cells
37.5	91.20701969
75	69.53576166
112.5	80.41220284
150	70.66829915
600	33.96796245

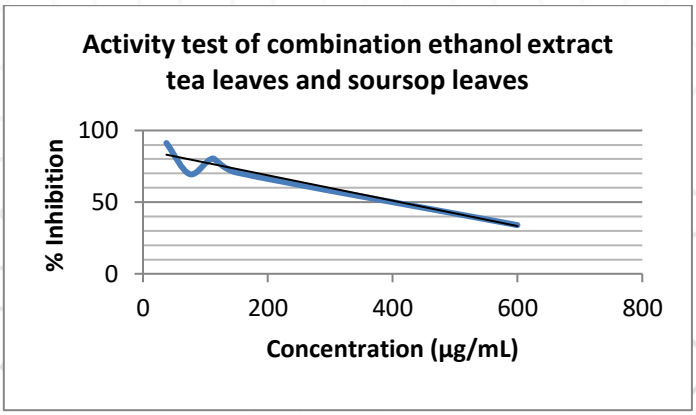


Figure 3. Graphic of linear regression combination of ethanolic extracts tea leaves and soursop leaves

In this study, by cytotoxic test the number of combination between ethanol extracts of lea and soursop leaf have a **strong potention** to inhibit WiDr cell cancer with the number of IC_{50} **41,375 µg/mL.**

CONCLUSION

The combination of ethanol extracts of tea leaves (*Camelia sinensis L.)* and soursop leaves *(Annona muricata l.)* based on DPPH method has IC_{50} value of **26.90 µg / mL** which is **very strong**. And based on the analysis in silico molecular docking using compound acetogenin in soursop leaves as having a strong potential of bcl-xl against cell with a docking score of **-6.7**. While the compound EGCG on leaves tea (*Camelia sinensis L.)* has a fairly strong potential with a docking score of **-8.1.**

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Effectiveness of Papaya Seeds (*Carica papaya L.*) and Turmeric (*Curcuma domestica val*) Juice Combination on Decreased of Triglyceride Levels on Animal Trials

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Abstract

Background: Cardiovascular disease has been the leading cause of death on world's population in recent years. Based on data, coronary heart disease and stroke are the biggest causes of death of 7.2 million people and 5.5 million people. The World Health Organization (WHO) and the World Heart Federation Organization (WHF) predict heart disease will be the main cause of death in Asian countries, especially in developing countries. Triglyceride levels above 200 mg/dL need to be considered and controlled. An increase in blood triglyceride levels of 1.0 mmol/L can increase the risk of ischemic heart disease by 14%. Papaya seeds and turmeric have antioxidant and hypolipidemia effect. **Aims**: This study to know the effect of papaya seeds (*Carica papaya L.*) and turmeric (*Curcuma domestica val*) juice combination to decreased triglyceride levels on animal trials.

Methods: This research is experimental laboratory with pre-test and post-test with control group design. The research subjects were 25 white rats (*Rattus novergicus*) male Wistar strain that divided into 5 groups: K(-) (negative control), K(+) (positive control), P1 (papaya seed juice of 100 mg / kgBW and turmeric juice of 70 mg/kgBW), P2 (papaya seed juice of 200 mg / kgBW and turmeric juice of 70 mg/kgBW), P3 (papaya seed juice of 400 mg / kgBW and turmeric juice of 70 mg/kgBW). The differences in triglyceride levels of pre-test and post-test were conducted by Oneway ANOVA test. The level of triglyceride was measured by *Enzymatic Colorimettric Test.*

Results: Significant differences and inhibition of increase of triglyceride levels (p = 0,000) is group P3 (papaya seed juice of 400 mg / kgBW and turmeric juice of 70 mg/kgBW).

Conclusion: Juice combination of papaya seeds (400 mg / kgBW) and turmeric (70 mg / kgBW) can effective to decrease triglyceride levels on animal trials.

Keywords : Papaya seeds, Turmeric, Triglyceride

INTRODUCTION

Cardiovascular diseases (CVDs) are the number one cause of death globally. An estimated 17.3 million people died from CVDs in 2008, representing 30% of all global deaths. Of these deaths, an estimated 7.3 million were due to coronary heart disease (*World Health Organization, 2011*). Hyperlipidemia is one of risk factors CVDs (Anwar, 2004). Changes in lifestyle by consuming fast food high in fat, high in calories and low in fiber and lack of physical activity can increase cholesterol levels, low density lipoprotein (LDL) and triglycerides (Ejaz, 2009).

Melinda P and Gurdita (2011) stated that administration of liquid papaya seed extract given for 30 days orally as much as 100-400 mg / kg / day can reduce triglyceride levels, total cholesterol, LDL cholesterol and VLDL cholesterol, and increase cholesterol levels HDL significantly in male wistar rats. Other plants that can reduce triglyceride levels are turmeric.

The provision of 1.4 grams of turmeric water extract can reduce lipid profiles such as total cholesterol, triglycerides, LDL, and VLDL in hyperlipidemic subjects for 90 days (Pashine *et al*., 2012). Administration of 500 mg of turmeric for 7 days orally can reduce lipid peroxidase levels (33%), total cholesterol (11.63%), and increase HDL cholesterol (29%) (Soni and Kuttan 1992).

In this study, the effectiveness of papaya seeds and turmeric juice combination was examined to find out the decrease triglyceride levels on animal trials

MATERIALS AND METHOD

The study was conducted at the laboratory of Pusat Studi Pangan dan Gizi Universitas Gadjah Mada and have obtained ethical approval from relevant ethics committee of faculty of medicine and health sciences Universitas Muhammadiyah Yogyakarta.

The tools are 25 mice cages, mats and cages, sonde, gloves, analytic scales, microhematocrit, test tube shelves, reaction drum, ependrofits, centrifuges, labels, timekeeper, vortex, pipette, blender, homogenizer, and filter. The material are aquabides, drinking water, standard food, papaya seed and turmeric juice, egg yolks.

Preparation of juice

The seeds of mature papaya fruit are mashed with a blender until smooth and then weighed to the desired level. After that, the distilled water was added to a volume of 1 mL, then homogenized using a homogenizer at a speed of 6000 rpm for 2 minutes. Turmeric that has been selected is pureed using a blender until smooth then weighed according to the desired level after that added distilled water to a volume of 1 mL, then in the hemogenizer at a speed of 6000 rpm for 2 minutes then filter.

Experimental animals

White rats (*Rattus novergicus*) male Wistar strain (25) selected based on inclusion and exclusion criterias (Federer, W. 2008). Inclusion criteria; the age of rat was 2 months, weighed ± 200 gram, healthy looking seen from the activity of movement. Exclusion criteria; visible anatomical abnormalities of rat, looked sick and do not move actively.

Experimental design

After 2 days of acclimatization period, the experimental animals were divided into five groups (n=5) as follows:

- **Group I/ K(-):** The animals received standard food and drinking water without tested juice and egg yolks for 21 days **(**negative control)
- **Group II/ (K+):** The animals are only induced by egg yolks for 21 days (positive control)
- **Group III/ P1:** egg yolks + papaya seed juice of 100 mg / kgBW and turmeric juice of 70 mg/kgBW for 21 days
- **Group IV/ P2:** egg yolks + papaya seed juice of 200 mg / kgBW and turmeric juice of 70 mg/kgBW for 21 days
- **Group V/ P3:** egg yolks + papaya seed juice of 400 mg / kgBW and turmeric juice of 70 mg/kgBW for 21 days

  All treatments were only orally administered continuously using a single dose of body weight. The effect of these groups on animal triglycerid levels were studied at the end of the study.

Biochemical analysis

Before and after the completion 21 days treatment, animals were fasted overnight and blood samples were collected from retro-orbital plexus. Blood samples were allowed to clot for approximately 1 hr at room temperature and centrifuged at 4000 rpm for 15 minutes to obtain the serum, used for estimation of triglyceride levels. The activities of these biochemical parameters were determined using commercially available kits (DiaSys Diagnostic Systems GmbH & Co. KG, Germany).

Statistical analysis

Data were expressed as mean ± SD (Standard Deviation). The statistical analysis was carried out by One Way ANOVA test. The value was statistically significant at $p<0.05$.

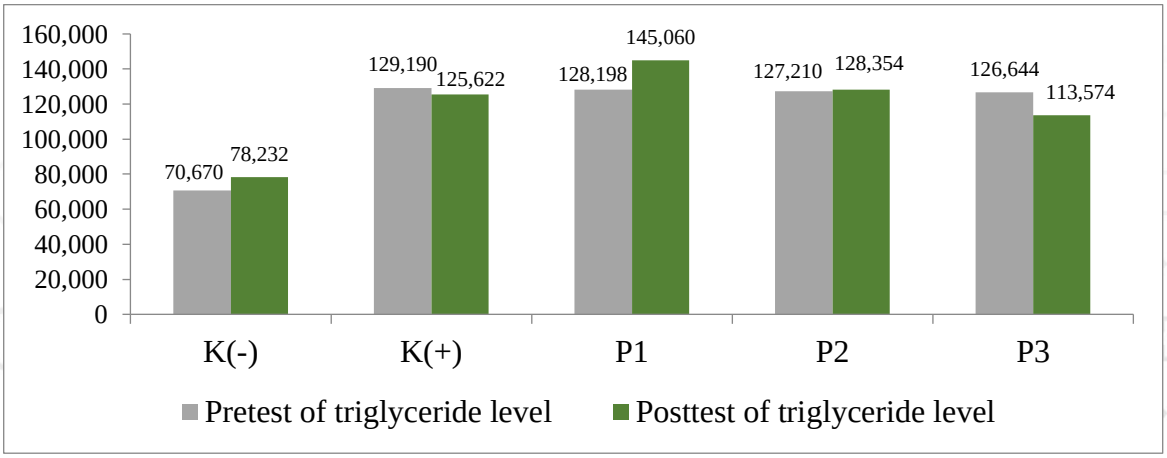
RESULT AND DISCUSSION

Mean difference of triglyceride levels in the pre-test and post-test groups using One Way ANOVA test. Table 1 shows there was a significant difference in the mean of triglyceride levels pre-test and post-test in K (-), P1, and P3 groups. The highest decrease of triglyceride levels was found in group P3.

Tabel 1. The mean of trigliceride levels in white rats *(Rattus novergicus)* Pre test and Post test Induction of papaya seeds and turmeric juice combination

Group	Triglyceride Level (mg/dL) ±SD		P value
	Pre-test	Post-test	(One Way ANOVA test)
K (-)	70.670±3.083	78.232±2.448	0.019
K (+)	129.190±2.974	125.622±5.640	0.228
P1	128.198±1.773	145.060±5.347	0.043
P2	127.210±2.063	128.354±3.381	0.631
P3	126.644±2.472	113.574±1.664	0.000

Data were expressed as mean ± SD (Standard Deviation). Data was significantly different if p value <0,05.



Grafic 1. The mean of trigliceride levels in white rats *(Rattus novergicus)* Pre test and Post test Induction of papaya seeds and turmeric juice combination

Based on Table 1 and Grafic 1,the highest decrease in triglycerides levels before and after the combination of papaya seed juice and turmeric juice in group P3 (p <0.05). This is different from Rahma studied (2013), who stated that there was no decrease in triglycerides due to consumption of papaya seed juice at a dose of 400 mg.

Papaya seeds contain flavonoids, saponins, tannins and alkaloids. Flavonoids and tannins can increase the activity of lipoprotein lipase so that it can reduce triglyceride levels in plasma (Do *et al*., 2010). Saponins can reduce fat absorption, triglyceride synthesis and increase fatty acid oxidation.

Turmeric contains active substances such as *atsiri oils* and curcumin compounds. *Atsiri oils* can reduce abdominal fat through regulation of peroxisome oxidation beta expression in the liver. *Atsiri oils* and curcumin also work in synergy in the regulation of genes that regulate fat metabolism (Honda, 2006). In mice that were given a diet high in cholesterol and turmeric there was a preventive increase in body weight and body fat due to decreased lipogenic expression in the liver (Shao, et al, 2012).

CONCLUSION

We conclude that juice combination of papaya seeds (400 mg / kgBW) and turmeric (70 mg / kgBW) can effective to decrease triglyceride levels on animal trials.

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Spiritual Intelligence Training to the Formation of Healthy Life Attitude

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Abstract

Background: Healthy conditions and illness can be determined from his attitude. The attitude of healthy life is influenced by the level of intelligence. Intellectual intelligence is only 20% acting in attitude, the rest there is another important role of intelligence is the spiritual intelligence.
Objective: to know the effect of spiritual intelligence training on the establishment of healthy life attitude of factory labor in Yogyakarta.
Method: This research uses experimental design with pre-post test control design method and collecting data by purposive sampling. Implementation of training at glove factory in Yogyakarta. The subjects were divided into 2 groups: control and treatment. The control group consisted of 10 respondents of factory labor who were not trained. The treatment group consisted of 10 respondents of factory labor who were given spiritual intelligence training for 5 days. Treatment groups were given 3 spiritual material sessions each day. Both groups before and after the training performed measurements of spiritual intelligence as well as a healthy lifestyle with questionnaire tools that have validated product moment and reliably tested Alpha Cronbach.
Result: t-test showed that spiritual score of respondent's intelligence after giving training there was a significant increase (p = 0,012). T-test analysis of respondents after the training there was an increase healthy life attitude score was significant (p = 0.047). Linear regression analysis showed a significant correlation (R = 861) increase in spiritual intelligence to increase healthy life attitude of employees of the plant.
Conclusions: spiritual intelligence can improve significantly healthier life stance on factory labor in Yogyakarta.

Keywords: spiritual intelligence, healthy life attitude, training

INTRODUCTION

Healthy attitude is very determining healthy life behavior. Unhealthy living attitudes result in unhealthy behavior. A healthy lifestyle becomes the basis for determining the quality of one's health. Factors affecting attitudes are personal experience, emotions of self and environment (culture, influence of others who are considered important, mass media, institutions or educational institutions, religious institutions, where someone is and so on) (Azwar, 2013). Healthy life attitudes play a role to direct action in the adequacy of nutritional needs, body activities, rest and behavior that became the basis of healthy lifestyles (Notoatmodjo, 2011). Unhealthy living attitudes result in low attention to quality of life (balance of nutritional needs, rest, physical activity and others). Work environment can affect healthy life attitude, if physical and non physical work environment is unhealthy, resulting in one's life attitude become unhealthy. The labor become ill from illness from their work environment (Kimura et al., 2005). Various work environments such as in factories or industries greatly affect the attitude of their labor, especially health problems (Li et al., 2009). Occupational pain was reported in the prevalence of hypercholesterolaemia (21.1%) in employees aged 20 years and over in 7 types of factories in Pulo Gadung industrial area of Jakarta (Bantas et al., 2012). Another case was submitted, in May 28, 2013 there has been an accident in the form of explosion of one of the pipes in the steam engine room Madukismo Sugar Factory Yogyakarta, causing 4 labor injured (Kristiawan, 2013). Cases of this kind can actually be prevented or minimized by various preventive efforts, among others, to build a healthy lifestyle with spiritual intelligence. Achievement of healthy life attitude can be influenced by self-management factor (Swaputri, 2010). Self-control with spiritual intelligence is shown in research, that the higher one's religiosity leads to lower free sex behavior (Andisti and Ritandiyono, 2008). Based on this condition, it is important to examine the spiritual intelligence training on the establishment of healthy living attitude of factory employees in Yogayakarta.

METHODS

This is an experimental study by providing training to improve spiritual intelligence and healthy lifestyle. The research model is Pre-Post-Test control design.
Respondents control group 10 people and group respondents who were given training spiritual intelligence 10 people. Determination of respondents with Purposive Sampling. Inclusion criteria in the form of permanent labor status with minimum 1 year working period. Permanent labor who suffer from mental illness or suffer illness in the care of doctors as exclusion criteria. Dependent variable in the form of healthy life attitude and independent variable in the form of spiritual intelligence. The respondents gave written informed consent prior to partisipation. Ethical approval was obtained from Faculty of Medicine and Health Sciences Universitas Muhammadiyah Yogyakarta Research Ethics Committee.

The data obtained is tabulated based on the filling of spiritual intelligence questionnaires and healthy living attitudes. Filling in this questionnaire is done before or after the provision of training of intelligence. This questionnaire measuring tool consists of 25 items of questions that have previously been tested for validity with product moment and reliability testing with Alpha Cronbach. The result of spiritual intelligence questionnaire test has r value count bigger than r table for n = 20 and α = 5% that is 0,444 so that item of question stated valid. The results of reliability test with Alpha Cronbach of 0.981 (meaning 0.981> 0.60) indicates that the variable of spiritual intelligence declared reliable. The condition of the validity test questionnaire validity of healthy life with r calculated 0.444 so it is said valid and test Reliability questionnaire attitude of healthy life with Alpha Conbrach of 0.763 (> 0.60) this condition indicates that the questionnaire is reliable.

The study was conducted for 4 months in the factory meeting room in Yogyakarta. Prior to the start of training, all respondents (control groups and treatment groups) measured their spiritual intelligence and healthy lifestyle. Education and training are given periodically with 4 levels. Each session takes 3 sessions, each session is 100 minutes long. Completed training, all respondents (control group and treatment group) measured spiritual intelligence and healthy life attitude. The data obtained were then tabulated and analyzed by One Sample Kolmogorov-Smirnov Test and Pair Sample Test.

RESULT

Data after analyzed by T-test (table 1) between spiritual intelligence on healthy life attitude showed a significant influence (P = 0.012). Linear regression analysis (table 2) shows the price of R = 0.861, meaning that the increase of spiritual intelligence significantly affects the healthy attitude of the respondent.

Table 1.
Tests of spiritual intelligence and respondents' healthy attitudes before and after training

Parameter	Pair Difference					t	df	Sig.(2-tailed)
	Mean	Std Deviation	std Error Mean	95% Confidence interval of the Difference Lower	Upper			
Spiritual Intelligence score Befor and after training	-9.60000	964019	304850	-16.49618	-2,70382	-3,149	9	0,012
Healthy Attitudes before-after training	-8.10000	12.28775	3.88573	-16.89013	-1.69013	-3.085	9	0,047

Table 2
Linear regression analysis healthy lifestyle attitudes against intelligence spiritual respondents

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	0,861 (a)	0,742	0,710	3,89173

Information : a Predictors (Constant), SI_after training,
SI = Spiritual intelligence

DISCUSSION

The mechanism of the process of improving the score of spiritual intelligence followed by the improvement of healthy life attitude (Tables 1 and 2) starts from the stimulation during the provision of education and training with the material of spiritual intelligence. The first material of this training is the concept of knowing themselves in the form of self-meaning; recognize the meaning of body, soul and spirit and recognize the meaning of life cycle of human life (Son et al., 2011). The provision of material in this training brings the stimulus to the respondent not only received by the physical senses but until received in the recipient system of excitatory components in his soul (Leo, 2011). Touch-induced stimuli are patterns of stimulation that can vibrate a person's soul and can be long-lasting in a person. Components of human composition consists of 2 parts of the physical and spiritual (Azwar, 2013). Meanwhile, based on the holy book of the Qur'an, man is composed of 3 components of the physical (physical), soul (soft body / software) and the soul (life). Stimulation in training is a touch of the heart, so it can enter up to the soul component. The stimulus of understanding the concept of self-meaning to respondents, this condition (Andisti *and Ritandiyono, 2008)* can strengthen the beliefs and positive expectations that cause respondents to be very enthusiastic and proactive in training. This is evident from the interaction of respondents who are very active in asking questions in the discussion so that the atmosphere of space becomes energized. The enthusiastic condition indicates that the respondent can receive the contents of the training materials well, so as to be able to accept new ideas, new opinions and new information about spiritual intelligence that has never been obtained. The ability of acceptance of something new is the main capital in making a change of attitude (Azwar, 2013) and (Singarimbun and Effendi, 1989). The next process, respondents are able to make changes from the old spiritual pattern to the perspective of the new concept of spirituality that can bring to make a flexible person by giving your self the ability without feeling restricted (Mahanggoro, et al., 2016). This condition is in accordance with the opinion of Zohar and Marshal (2000) which states that increased spiritual intelligence makes a person a creative person, capable of distinguishing a good and bad behavior. The ability to distinguish the virtues and ugliness of this behavior that causes self-awareness of respondents to a sense of accountability to God in various aspects of life including in accounting for his body. The awareness is responsible for this body that fosters the healthy attitude of the respondent to be well formed. This sense of responsibility includes the awareness of keeping the diet, the activity of fiscal motion and control to rest. This condition of spiritual enhancement that plays an important role in the formation of a healthy attitude of respondents.

CONCLUSIONS

Spiritual intelligence and healthy living attitudes of labor increased significantly after training. Improvement of spiritual intelligence can form a healthy living attitude of factory labor in Yogyakarta.

Conflict of interests

The author declare that they have no conflicting interests related to manuscript.

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The Evaluation on Antibiotic Use in Patients With Typhoid Fever in Inward Installation of Hospital X

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Abstract

Typhoid fever is an infectious disease caused by *Salmonella typhi* bacteria. In 2011, the proportion of hospitalized patients with typhoid fever and other gastrointestinal diseases in the Yogyakarta Special Province reached 11,536 cases. One of the causes of less optimum treatment of typhoid fever is the emergence of Multidrug Resistant *Salmonella typhi* strain. Evaluation of antibiotic use was carried out by standardized methods, namely ATC / DDD and DU 90%. This study aims to determine the profile of antibiotic use and evaluation of antibiotics in hospitalized patients with typhoid fever in PKU Muhammadiyah Bantul Hospital in 2015.

The study applied a non-experimental research with descriptive analytic design. Sample data were 30 patients. Analysis of antibiotic use was carried out using the ATC / DDD method, DU 90%, and its compatibility used Guidelines for Management of Typhoid Fever issued by WHO in 2011.

The results of this study indicated that the total use of antibiotics in 2015 reached 105.2 DDD / 100 patient-days, and ceftriaxone was the most widely used antibiotic which was equal to 60.8 DDD / 100 patient-days. Ceftriaxone, levofloxacin and azithromycin were included in DU 90%. The percentage of suitability on antibiotic based on Guidelines for Management of Typhoid Fever issued by WHO in 2011 was 97%.

Keywords : Antibiotics, Typhoid Fever, ATC / DDD, 90% DU

INTRODUCTION

Typhoid fever is an acute infection of the small intestine with fever symptoms more than one week, resulting in digestion problem and reducing the level of consciousness[1]. Typhoid fever caused by *Salmonella typhi* bacteria happens globally[2].

Typhoid fever ranked third out of the top 10 most diseases in hospitalized patients in Indonesia with 41,081 cases (Case Fatality Rate (CFR) = 0.55%). The first rank was occupied by diarrhea and gastroenteritis with 71,889 cases (CFR = 1.79%), and the second place was occupied by DHF with 50,115 cases (CFR = 0.67%) [3]. According to the health profile of Yogyakarta Special Region in 2011, typhoid fever and other gastrointestinal infections were the most dominant cases in hospitalized patients in Yogyakarta Special Province with a total of 11,536 new cases[4].

Typhoid fever has not been managed optimally for several reasons, including the emergence of Multidrug Resistant *Salmonella typhi* strains, the increase of career cases and relapse, the difficulty to make effective vaccines and the widespread of irrational use of drugs. Those aspects often cause ineffective treatment and trigger the patients to repeat the treatment or replace the drug so that it requires greater costs [5]. Studies conducted in 2010 in five countries in Asia (China, India, Indonesia, Pakistan and Vietnam) which became the endemic to typhoid fever reported a prevalence of multidrug-resistant typhoid fever ranging from 7% to 65% [6].

Evaluation of drug use must be carried out continuously using a standardized system or method [7]. Since 1996, WHO has recommended the classification of ATC (Anatomical Therapeutic Chemical) along with DDD (Defined Daily Dose) unit as a global standard for the study of drug use and reporting of drug effect reactions. Thus, this system has been widely used internationally[8]. DU 90% (Drug Utilization 90%) is used to explain the pattern of drug use by grouping drug data used for qualitative assessment as well as for international comparisons between drug use and prescribing patterns by doctors[9].

PKU Muhammadiyah Bantul Hospital is a private hospital which becomes one of the references in Bantul Regency with a large number of typhoid fever patients hospitalized. Based on this background the researchers were interested to evaluate the use of antibiotics in typhoid fever patients in the Inpatient Installation of PKU Muhammadiyah Bantul Hospital in 2015 using the ATC / DDD method, 90% DU and their suitability for the guidelines for treating typhoid fever based on the Guidelines for Typhoid Fever Management issued by WHO in 2011.

MATERIALS AND METHOD

1. Materials

The instruments applied in the study were medical records of typhoid fever patients in the Inpatient Installation of PKU Muhammadiyah Bantul Hospital in the period of

January to December 2015. ATC codes and DDD units were accessed through the official website of WHO Collaboration Center and guidelines for typhoid fever treatment based on Guidelines of the Management for Typhoid Fever issued by WHO in 2011.

2. Methods

The study applied an observational study with retrospective data collection obtained from medical records of typhoid fever patients in the Inpatient Installation of PKU Muhammadiyah Bantul Hospital in the period of 2015. The data were be presented descriptively. Analysis of drug use was carried out by using the ATC / DDD method, DU 90% and analyzed its suitability with the guidelines for treating typhoid fever based on the Guidelines of the Management for Typhoid Fever issued by WHO in 2011.The research data were taken from medical records of 30 typhoid fever patients who were hospitalized in PKU Muhammadiyah Bantul Hospital in 2015, and the respondents should meet the inclusion criteria.

This research covered several step namely writing proposal, making ethical permission, and selecting samples that were included in the inclusion criteria. Data on antibiotic use were then evaluated using the ATC / DDD method, 90% DU and antibiotic suitability for the guidelines for the treatment of typhoid fever based on the Guidelines of the Management for Typhoid Fever issued by WHO in 2011.

3. Data Analysis

The data of Antibiotic used from the research subjects were classified based on the ATC code and DDD units accessed through the official WHO Collaboration Center website[11]. The amount of antibiotic use that owned ATC codes (in grams) was converted into a DDD unit with the formula:

$$\text{DDD use} = \frac{\text{Grams of antibiotic}}{\text{DDD standard based on WHO}}$$

DDD/100 patient-days were then counted with this equation:

$$\frac{\text{Amount of antibiotic used by the patients}}{\text{DDD standard based on WHO}} \text{ x } \frac{100}{\text{total } \textit{length of stay} \text{ (LOS)}}$$

The antibiotics used as the treatment of typhoid fever were sorted by the percentage of use in the form of % DDD from the highest to the lowest, then determined the type of antibiotics that could be included in 90% DU. The criteria are compiled from the Guidelines of the Management for Typhoid Fever issued by WHO in 2011. Data that had been processed and grouped into frequencies and percentages were analyzed descriptively then presented in form of table.

RESULT AND DISCUSSION

A. Characteristics of Patients

1. Patient Distribution by Gender

The distribution of typhoid fever patients by sex in PKU Muhammadiyah Bantul Hospital in 2015 showed that there were 20 women (67%) and 10 patients (33%) of 30 patients with typhoid fever. The results of the research on the distribution of patients by sex can be seen in Table 1.

Table 1. Patient Distribution by Gender

Sex	Amount	%
Males	10	33
Females	20	67
Total	**30**	**100**

The results of this study showed that it was in line with the results presented by Health Ministry of Indonesian Republic (2011) which explained that typhoid fever was found to be more prevalent among women than men. A research result by Rustam (2011) in Saewangan Maros Regional Hospital also showed that typhoid fever patients were more women (56.1%) than men (43.9%).

2. Patient Distribution by Age

Patient distribution by age in this study consisted of patients aged 18-29 years, 30-39 years, 40-49 years, 50-59 years and ≥60 years. The incidences of typhoid fever in adults can be due to eating snacks outside the home, not washing hand using soap before eating, having a history of typhoid fever and lacking of clean water in everyday use[13]. The results of the study of the distribution of typhoid fever patients in PKU Muhammadiyah Bantul Hospital in 2015 based on age can be seen in Table 2.

Table 2. Patient Distribution by Age

Age (year)	Numbers (people)	%
18-29	9	30
30-39	6	20
40-49	7	23
50-59	6	20
≥ 60	2	7
Total	**30**	**100**

The results of the study in Table 4 show that the age group of 18-29 years was the most dominant age group that had the highest typhoid fever with a percentage of 30%; the second one was age groups 40-49 with a percentage of 23%; the third was the age group 30-39 years; the next was 50-59 years with percentage of each 20%, and the last age group > 60 with a percentage of 7%. These results of the study were in line with the research at Salewangan Maros General Hospital showing that there was a relationship between age groups and the incidence of typhoid fever in hospitalized patients in which the percentage of typhoid fever patients in the age group of 20-29 years reached 23.5%, the largest group among to other age groups[12].

B. Profile of Antibiotics Use

The data of antibiotics given for typhoid fever patients in inpatient installation at PKU Muhammadiyah Bantul Hospital in 2015 were obtained from medical record data in antibiotic types, antibiotic doses, route of administration and amount of use in each patient. The antibiotics used were from the first generation cephalosporin group (cefadroxil), third generation cephalosporins (cefixime and ceftriaxone), macrolides (azithromycin) and floroquinolone (levofloxacin). Antibiotics used in typhoid fever patients in the Inpatient Installation of PKU Muhammadiyah Bantul Hospital in 2015 can be seen in Table 3.

Table 3. Profile of Antibiotic Use in Typhoid Fever Patients in Inpatient Installation of PKU Muhammadiyah Bantul Hospital in 2015

Antibiotics	Dosages	Prescription Numbers	%
Azitromycin	500 mg 1x a day	2	6
Cefadroxil	500 mg 2x a day	1	3
Cefixime	100 mg 2x a day	1	3
Ceftriaxone	1000 mg 1x a day	18	55
Levofloxacin	500 mg 1x a day	11	33
Total		33	100

Based on the data in Table 3, the most widely used antibiotics for typhoid fever patients in the Inpatient Installation of PKU Muhammadiyah Bantul Hospital in 2015 were third generation cephalosporins, namely ceftriaxone as many as 18 prescription (55%). Ceftriaxone is able to reduce body temperature to normal significantly so that it can be the drug of choice for patients with typhoid fever[14]. The second most used antibiotic was levofloxacin as many as 11 prescriptions (33%). Levofloxacin had 100% clinical efficacy for the treatment of typhoid fever[15].

Azitromycin from the macrolide group was the third most used antibiotic as many as 2 prescriptions (6%). Azitromycin was effective, both clinically and bacteriologically for typhoid fever caused by *Salmonella typhi* and multidrug resistant *Salmonella typhi* [16]. Furthermore, cefadroxil (first generation of cephalosporins) and cefixime (second generation of cephalosporins) were one recipe each. Cefadroxil is included in the national formulary used in Universal Health Coverage [17]. On the other hand, cefadroxil has not been proven effective for typhoid fever and has significantly reduced fever. Cefixime is the antibiotic choice if there is an indication of leukocytes decrease until <2000 / µl or resistance to *Salmonella typhi* [18].

C. Evaluation of the Antibiotics Use

1. ATC / DDD

Evaluation of antibiotic use in typhoid fever patients in the inpatient installation of PKU Muhammadiyah Bantul Hospital in 2015 was carried out using the ATC / DDD method. The antibiotics were sorted according to the ATC code based on the WHO Guidelines Collaborating Center of Drug Statistic Methodology. The quantity of antibiotic use in the study was then calculated using a DDD measurement unit with DDD /100 patient-days.

DDD values based on the WHO Guidelines Collaborating Center of Drug Statistics Methodology can be called as definitive DDD meaning as one strength per patient. In addition, to get DDD, the study was conducted by dividing the total use in units of milligrams with definitive DDD in units of milligrams/patient. After the DDD results were obtained then the DDD / 100 patient-days were calculated. DDD/100 patient-days described how many patients received DDD antibiotics for patients with typhoid fever. DDD/100 patient-days were calculated by using DDD multiplied by 100 patient-days divided by the length of stay (LOS) from 30 patients, 125 days. Quantitative data of antibiotic use can be seen in Table 4.

Table 4. Quantity of DDD Antibiotic Use in Inpatient Installation of PKU Muhammadiyah Bantul Hospital in 2015

ATC Code	Antibiotics	Total Use (mg)	Definitive DDD WHO (mg/patiet)	Total *Length of Stay* (n=30)	DDD/100 *patient-days*
J01FA10	Azitromycin	4500	300 (O)		12
J01DB05	Cefadroxil	2000	2000	125	0.8
J01DD08	Cefixime	600	400		1.2

J01DD04	Ceftriaxone	152000	2000	60.8
J01MA12	Levofloxacin	19000	500	30.4
Total				**105.2**

The greater value of DDD/100 patient-days indicated a high level of antibiotic use. Based on the data in Table 4, it can be seen that the evaluation results of the antibiotic use in typhoid fever patients in PKU Muhammadiyah Bantul Hospital in 2015 reached 105.2 DDD/100 patient-days with ceftriaxone as the most widely used antibiotic which DDD/100 patient- days was 60.8. It means that in 100 days of treatment there were 60-61 patients receiving ceftriaxone therapy with 2000 mg per day. Susilo (2011) stated that typhoid fever patients in the Inpatient Installation of PKU Muhammadiyah Surakarta Hospital in 2010 reached 57.68 DDD/100 patient-days as the use of ceftriaxone.

Levofloxacin became the second most dominantly used antibiotic in typhoid fever patients in PKU Muhammadiyah Bantul Hospital in 2015 with 30.4 DDD/100 patient-days. The results of research conducted by Nelwan (2012) showed that the effectiveness of levofloxacin in typhoid fever was 100% with minimal side effects.

The third most widely used antibiotic for typhoid fever patients in PKU Muhammadiyah Bantul Hospital in 2015 was azithromycin with the value of 12 DDD / 100 patient-days. According to Frenck et al., (2004) treatment with oral azithromycin for typhoid fever cases of children showed no relapse.

Cefixime was used as much as 1.2 DDD / 100 patient-days for typhoid fever patients in PKU Muhammadiyah Bantul Hospital in 2015. The use was lower compared to the use of cefixime for typhoid fever patients in PKU Muhammadiyah Surakarta Hospital in 2010 as much as 28.125 DDD/100 patients –days[18]. The lowest use of antibiotics for typhoid fever patients in PKU Muhammadiyah Bantul Hospital in 2015 was cefadroxil by 0.8 DDD/100 patient-days which means that among 100 patients there was only one patient who received cefadroxil therapy at 2000 mg per day.

2. DU 90%

Drug Utilization 90% (DU 90%) was applied to explain the pattern of drug use by grouping drug data which had been used for qualitative assessment as well as for international comparisons based on 90% of the whole drugs used [9]. Antibiotics that could be categorized to the DU segment 90% were drugs that were included in the accumulation of 90% after being sorted. DU 90% used for typhoid fever patients in the Inpatient Installation of PKU Muhammadiyah Bantul Hospital in 2015 can be seen in Table 5.

Table 5. DU 90% of Antibiotics Used for Patients with Typhoid Fever in Inpatient Installation at PKU Muhammadiyah Hospital in Bantul in 2015

ATC	Antibiotics	DDD/100 *patient-days*	% Cummulative	Segment
J01DD04	Ceftriaxone	60.8	57.8	DU 90%
J01MA12	Levofloxacin	30.4	86.7	
J01FA10	Azitromycin	12	98.1	
J01DD08	Cefixime	1.2	99.2	DU 10%
J01DB05	Cefadroxil	0.8	100	

Based on the cumulative percentage, it can be seen that there are three types of antibiotics which were included in the 90% DU namely ceftriaxone, levofloxacin and azithromycin for typhoid fever patients in PKU Muhammadiyah Bantul Hospital in 2015. Antibiotics for the treatment of typhoid fever in the DU 10% segment must be replaced with antibiotics which are included in the DU segment 90% [17].

3. Conformity to the Antibiotics Use based on Therapeutic Guidelines

Antibiotics use for typhoid fever patients in the Inpatient Installation of PKU Muhammadiyah Bantul Hospital in 2015 were five types, namely azithromycin, cefadroxil, cefixime, ceftriaxone and levofloxacin. All of these antibiotics were seen the consistency with the guidelines for treating typhoid fever based on the Guidelines for the Management of Typhoid Fever issued by WHO in 2011. The suitability of antibiotic use can be seen in Table 6.

Antibiotics	Therapy Guidance	Prescription Numbers	%
Azitromycin	✓	2	6
Cefadroxil	-	1	-
Cefixime	✓	1	3
Ceftriaxone	✓	18	55
Levofloxacin	✓	11	33
Total			**97**

Table 6. Suitability of Antibiotics Use in Typhoid Fever Patients in Inpatient Installation of PKU Muhammadiyah Bantul Hospital in 2015 based on Guidelines for the Management of Typhoid Fever issued by WHO in 2011.

The use of antibiotics in accordance with the treatment guidelines for typhoid fever based on the Guidelines for Management of Typhoid Fever issued by WHO in 2011 reached 97% namely two prescriptions (6%) of azithromycin, three prescriptions (3%) of cefixime, ceftriaxone (55%) and 11 precriptions (33%) of levofloxacin. Azitromycin was effective, both clinically and bacteriologically for typhoid fever caused by *Salmonella typhi* and multidrug resistant *Salmonella typhi* [16]. Ceftriaxone had been proven effective for treating typhoid fever patients [16]. Cefixime was often used as an alternative if there was indication of a decrease in leukocyte value up to <2000 / µl or resistance to *Salmonella typhi* [18]. According to Nelwan et al. (2006), levofloxacin had 100% clinical efficacy for the treatment of typhoid fever.

There was only one antibiotic type that was not suitable namely cefadroxil. Cefadroxil was included in the national formulary used in Universal Health Coverage [17]. However, cefadroxil had not been proven effective for typhoid fever and significantly reduced fever. High level suitability (97%) showed that in PKU Muhammadiyah Bantul Hospital in 2015 the use of antibiotics for typhoid fever had been carried out according to the Guidelines for the Management of Typhoid Fever issued by WHO in 2011.

CONCLUSION

The most widely used antibiotic for typhoid fever patients in the Inpatient Installation of PKU Muhammadiyah Bantul Hospital in 2015 was respectively ceftriaxone with 18 prescriptions (55%), levofloxacin as many as 11 prescriptions (33%), azitromycin in two prescriptions (6%) and then cefadroxil and cefixime as many as one recipe (3%).The evaluation results of antibiotic use in typhoid fever patients in the Inpatient Installation of PKU Muhammadiyah Bantul Hospital in 2015 reached to 105.2 DDD/100 patient-days with ceftriaxone as the most widely used antibiotic with 60.8 DDD/100 patient-days, levofloxacin with 30.4 DDD/100 patient-days, azithromycin at 12 DDD/100 patient-days, cefixime at 1.2 DDD/100 patient-days and cefadroxil at 0.8 DDD / 100 patient-days. Three antibiotics namely ceftriaxone, levofloxacin and azitromycin could be included in the DU 90% segment. Percentage of antibiotic use suitability with the treatment guidance for typhoid fever based on the Guidelines for Management of Typhoid Fever issued by WHO in 2011 was 97%.

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The Evaluation of Antibiotics Use Rationality in the Treatment of Patients With Urinary Tract Infection at the Inpatient Installation Ward in RSUD Kab Temanggung Period of January-December 2015

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Abstract

Urinary tract infection is one of the most infectious diseases suffered by inpatients in RSUD Kabupaten Temanggung. Antibiotic is a therapy option for urinary tract infections (UTIs) disease. The inappropriate use of antibiotics is one of the factors causing high rates of microorganism resistance to antibiotics. This study aimed to evaluate the rationality of antibiotics used for UTIs treatments of medical patients in RSUD Kabupaten Temanggung period of January-December 2015.

This study was descriptive analytic with retrospective method based on the data that were taken from the patient's medical records. The samples of this study were patients with UTIs diagnose and antibiotic for their therapy in RSUD Kabupaten Temanggung period of January-December 2015. The sampling method used purposive sampling method including a non-probability sample method. The sampling was based on the researcher's consideration which contains chosen substances (based on specific inclusion criteria).

This study was conducted on 74 patients who met the inclusion criteria with the proportion of 63, 52% female and 36, 48% male. There were six kinds of antibiotics therapy such as ceftriaxone (50%), ciprofloxacin (31, 08%), cefotaxime (8, 11%), amoxicillin (5, 41%), levofloxacin (2, 70%), and cefepime (2,70%). In this study, the rationality of antibiotic use could be seen from right indication (100%), right drug (100%), and right dose (87, 84%). Thus, it is concluded that the rationality of antibiotics use were 65 patients (87, 84%) from 74 samples which have been studied.

Keywords : Antibiotics, urinary tract infection, rationality

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INTRODUCTION

Urinary tract infection is one of the most infectious diseases suffered by inpatients in RSUD Kabupaten Temanggung (Temanggung District Hospital). The data taken from the Central Bureau of Statistics of Temanggung Regency in 2014 showed that RSUD Kabupaten Temanggung treated UTIs patients with the biggest number compared to the other hospitals in Temanggung. It treated 241 UTIs patients.

Based on the guidelines for the treatment of Infection at the Genital and Urinary Tracts, the severity level and the place of infection are the base to choose the kind of antibiotics. Drug dose, administration route, and duration of the administration become the material to considering the antibiotics used for effective therapy. The number of infections which occurs makes antibiotics to be the most given drug to the patients. In the developing country, 30-80% of inpatients are given antibiotics.

Based on the percentage, 20-65% of the antibiotic therapy is incorrect. The incorrect use of antibiotics by paramedic can cause antibiotic resistance and unwanted drug effect (Lestari et al., 2011).

MATERIALS AND METHOD

Research Design

This research is a cross-sectional descriptive, i.e., collecting data (observation) at one time. The data were taken retrospectively by recording the data through the patient medical record.

Population and Sample

Population

The population in this research is all the UTIs inpatients in RSUD Kabupaten Temanggung, period of January - December 2015.

Sample

The sample of this research was taken from the population which meets the inclusion criteria. The sample taking was based on the researcher's consideration containing the chosen substances (based on specific inclusion criteria).

Inclusion

The inclusion criteria are all the patients with UTIs as the final diagnose without complication/comorbidities. They were given antibiotic therapy and treated in the inpatient installation ward of RSUD Kabupaten Temanggung, period 2015.

Exclusion

The exclusion criteria are the incomplete data of the patient medical record, the patient who stop the treatment based on their request and the patient who died during the therapy period.

Research Instrument

Medical Record

The instrument of this research is the medical record data of inpatient with the diagnose of UTI in RSUD Kabupaten Temanggung, completed with the laboratory data as a supporting diagnosis.

Guideline of UTIs Treatment

The guideline used to evaluate the rationality of the antibiotics used in this study is the guidelines of the treatment of Infection at the Genital and Urinary Tracts and the guidelines of Medical Service by Indonesian Pediatrician Association in 2009 (Ikatan Dokter Anak Indonesia Tahun 2009).

Place and Time

This study was conducted in RSUD Kabupaten Temanggung which located on Dr. Sutomo Street No.67 Temanggung, Jawa Tengah, Indonesia. The data were taken in Juni-Juli 2016.

Data Collection Procedure

a. The data collection of the number and the medical record number of the patients with UTIs diagnose in the inpatient installation ward of RSUD Kabupaten Temanggung, period of January- December 2015.
b. The selection of UTI patients medical record which meets the inclusion criteria in the period of January-December 2015.
c. Recording the data in the data collection sheets.
d. Evaluate the rationality of antibiotic use based on the guidelines or therapy standard.
e. Determine the result, discussion, and conclusion.

RESULT AND DISCUSSION

Rationality analysis was conducted by observing the use of antibiotics in each case. Then, it was compared to the guidelines or the therapy standard which was used as the

treatment reference, including:

a. Right Indication, i.e., chose the right drug which is given in accordance with the doctor's diagnosis supported by the laboratory result and clinical symptoms. Right indication is based on the guidelines of the treatment of Infection at the Genital and Urinary Tracts
b. Right drug, i.e., chose the right antibiotics given to the patient based on the guidelines for the treatment of Infection at the Genital and Urinary Tracts

The patient's characteristics in this study are based on gender, age, symptoms, and the duration of the therapy.

The number of woman UTI patient is 47 people with the percentage of 63, 52% and the number of man UTI patient is 27 people with the percentage of 36, 48%. This is due to the short female urethra and it always contains germs. As much as 50% women will have UTI during their lifes (Rasjidi, 2013).

Patients Characteristics Based on Age

In this study, UTI infected women mostly happen in the age range of 26-35 years old with the percentage of 25, 53%. Meanwhile, for the man, UTI mostly infects men with the age range of 36-45 years old, 46-55 years old, and 56-65 years old with the same percentage of 18, 51%.

The Guidelines for the Treatment of Infection at the Genital and Urinary Tracts explain that the woman who is in the sexually active phase has high risk of being infected with UTI. The age range is 20 to 40 years old. Several data of the man show the increase of UTI as they get older, but its prevalence is always under the woman's prevalence in the same age range (Sukandar, 2009).

The patient's Characteristics Based on The Complaints

Table 1. Clinical symptoms of UTI patient

No	UTI Clinical Symptoms	Total
1	Fever	24
2	Low back pain	7
3	Abdominal pain	30
4	Impaired Urination	21

In this study, urination is divided into three, namely felt hot urination, painful urination and, the number and frequency of the urination. Those symptoms are in accordance with *The Guidelines for the Treatment of Infection at the Genital and Urinary Tracts* which stated that clinical symptoms of UTI are dysuria, back pain, pelvic pain, and fever.

The sample's Characteristic Based on Therapy Duration

Table 2. Therapy Duration of UTI Patients

Therapy duration (day)	Total	Percentage
1	8	10,81%
2	18	24,32%
3	28	37,84%
4	9	12,16%
5	7	9,46%
6	2	2,70%
7	1	1,35%
8	1	1,35%
Total	**74**	**100%**

The longest therapy duration is three days; it is 37, 84%. It is in accordance with *The Guideline for the Treatment of Infection at the Genital and Urinary Tracts* which stated that the recommended empirical medication to the UTI patient without complication is for three days. The medication for more than three days doesn't provide the same effectiveness; it increases the number of the complication.

Antibiotics for the UTIs Patients

Table 3. The Antibiotics Given to the UTIs Patients

Antibiotics	Total	Percentage
Ceftriaxone (IV)	37	50%
Ciprofloxacin (per oral)	23	31,08%
Amoxillin (per oral)	4	5,40%
Levofloxacin (IV)	2	2,70%
Cefotaxime (IV)	6	8,11%
Cefepime (IV)	2	2,70%
Total	**74**	**100%**

The antibiotic used for UTI therapy is a single antibiotic. Most of the antibiotics were given through the intravenous route (47 patients), and the other is in per oral way (27 patients). From the six antibiotics above, the most widely used is ceftriaxone.

Rationality Evaluation of Antibiotics Use

The evaluation of antibiotic use to 74 UTI patients in the inpatient ward of *RSUD Kabupaten Temanggung* use WHO indicator year of 1985, i.e., right indication, right drugs, and right doses. This evaluation was conducted by comparing the therapy given to the patients to the therapy standard based on *The Guidelines for the Treatment of Infection at the Genital and Urinary Tracts.* For the pediatric patient, it uses *The Guidelines for Medical Service of Indonesian Pediatric Association in 2009.* The result of this evaluation is provided in the table below.

Table 4. The Rationality of Antibiotics Use on the UTI Patients in the Inpatient ward of RSUD Temanggung Regency Period of January-December 2015

	Right Indication	Right Drug	Right Dose	Conclusion	
				Proper Use	Improper Uses
The number of patients	74 (100%)	74 (100%)	65 (87,84%)	65 (87,84%)	9 (12, 16%)

Table 4 shows that from the 74 samples, all of them (100%) meets the criteria of right indication and right drug. Meanwhile, the right dose is as many as 65 patients (87, 84%).

Right Indication

The evaluation of right indication was based on the doctor's diagnosis. The diagnosis was made by physical examination, clinical symptoms, and laboratory examination. The infection from the urethra (urethritis) and bladder (cystitis) usually shows the symptoms in the form of the combination of frequency, urgency, isuria, pyuria, hematuria, acute or chronic pelvic pain, back pain, or fever (Rasjidi, 2013). The administration of antibiotics to the 74 UTIs patients who had been studied was in accordance with the indication. There were 31 samples experiencing an increase in the number of leukocytes. The other samples (43 patients) were based on the clinical symptoms in the form of fever, back pain, dysuria, hematuria, leukocytosis, lower back pain, lower abdominal pain, and urination with the pain and hot feeling.

Right Drug

The selection of antibiotics for the treatment of UTI patients in the inpatient installation ward of Temanggung Regency Hospital is right. This antibiotic selection was in accordance with the used Guidelines, namely *The Guidelines for the Treatment of Infection at the Genital and Urinary Tracts.* Five pediatric patients were treated with cephalosporin-class antibiotics. The selection of ceftriaxone and cefotaxime for the pediatric patients of UTI therapy was in accordance with *The Guidelines for Medical Service of Indonesian Pediatric*

Association in 2009.

Right Dose

The right dose evaluation was divided into three parameters, namely right dose dosage, right frequency of administration, and right route of antibiotics administration. Based on *The Guidelines for the Treatment of Infection at the Genital and Urinary Tracts,* the dose of intravenous ceftriaxone is 1-2 gram/day, ciprofloxacin peroral dose is 2x500 – 750 mg/ day, amoxicillin peroral dose is 3x250 – 500 mg/day, intravenous levofloxacin dose is 250 – 750 mg/day, intravenous cefotaxime is 1-2 gram twice a day, and the dose of intravenous cefepime is 1-2 gram twice a day. Nine patients were concluded given improper dosage or the number of antibiotics based on the therapy standard used. Those 9 patients consist of 4 pediatric patients who treated with high-dose ceftriaxone, two patients with high-dose amoxicillin, two patients with high-dose levofloxacin, and one patient with underdose cefotaxime.

The parameter of right dose based on the frequency of the administration had a result that one from the 74 patients was not in the category of right drug. This patient was an adult patient who received 500 mg of amoxicillin every 6 hours. Based on the therapy standard used as a reference, 500 mg of amoxicillin should be given every 8 hours.

The third parameter of right dose is the antibiotics administration route. All of the 74 patients were in right route of antibiotics administration. Thus, it can be concluded that the evaluation of the right dose given as therapy is 65 patients (87, 84%).

CONCLUSION

1. Antibiotics prescribed by doctors were ceftriaxone (50%), ciprofloxacin (31.08%), cefotaxime (8.11%), amoxicillin (5.41%), levofloxacin (2.70%), and cefepime (2.70 %).
2. The use of antibiotics that meets the exact criteria of right indication (100%), right drug (100%), and right dose (87.84%). So that, the rationality of antibiotic use was 65 patients (87.84%), while the inappropriate ones were 9 patients (12.16%) out of a total of 74 patients.

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Correlation of Ki-67 Expression with Histopathologic Characteristics of Triple Negative Breast Cancer

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Abstract

Triple negative breast cancer is one of the breast cancer with poorest prognosis. Ki- 67, grade and lymphovascular invasion are the prognostic pathomolecular parameter, and also use as guiding for adjuvan therapy. The objective of this study is to identify the relationship between Ki-67 expression with grade and lymphovascular invasion in triple negative breast cancer. We found 19 cases of triple negative breast cancer,and performed slide review for grade and lymphovascular invasion. The results of Ki-67 expression were classifiy as low – intermediate (< 10 % - 29%) and high expression (> 30%). We use Chi Square test to assest the relationship between variables. From 19 triple negative breast cancer cases, we found 52,6 % of cases with high histopathological grade. Positive lymphovascular invasion is seen in 68,4% cases. We only found 1 case with high expression of Ki-67. There were no correlation between between Ki-67 expression with histopathological grade and lymphovascular invasion on triple negative breast cancer. There were no significant relationship between Ki-67 expression with grade and lymphovascular invasion on triple negative breast cancer.

Keywords : Triple Negative Brest Cancer, Ki-67, grade, LVI

INTRODUCTION

Breast carcinoma is the most common malignancies in women of North America, Western South Europe and Australia. In the United States, about 100,000 new cases are diagnosed each year and about 30,000 patients die from the disease. Based on data from the Cancer Registry Agency of Indonesian Pathology Specialist Association in 2006, breast carcinoma in Indonesia ranked second most malignant tumors in women after cervical carcinoma but increased in 2009 to rank first with 4,130 cases (18.19%). Breast carcinoma ranks first in West Sumatra, with an increasing number of cases each year (BRK IAPI, 2006; BRK IAPI, 2009).

Breast carcinoma is the leading cause of death from cancer in women aged 35-55 years. The disease is heterogeneous both clinically and pathologically so it is difficult to estimate survival because the degree of malignancy varies greatly, as does the patient's response to treatment. The 5-year survival rate is estimated at about 65%, with a large difference in each stage (Rosen, 2009; Uzzan et al, 2004).

Various studies have been done to find the parameters of prognosis in breast cancer, either clinically, pathologically or molecularly. Some known prognostic parameters include patient age, tumor size, lymph node status, histologic degrees of differentiation, histologic subtype, vascular invasion and estrogen receptor status (Uzzan et al, 2004). Recent WHO classifications (2012), known subtypes include invasive carcinoma of no special type (formerly known as invasive ductal carcinoma), invasive lobular carcinoma, medullary carcinoma and so on (Colditz et al, 2012).

From previous studies it was found that breast cancer with the same histologic parameters could show different biomolecular profiles.(Lester, 2015). Therefore molecular classification of breast carcinoma was established based on estrogen receptor status, progesterone receptor, Her-2 / neu expression and Ki-67, consisting of luminal A, luminal B, Her-2 positive and triple negative. Of these 4 types, the luminal A has the best prognosis and the triple negative is the subtype with the worst prognosis (Cheang et al, 2011; Shomaf et al).

Triple negative breast cancer compared to other types is aggressive because it grows faster, is more likely to metastasize and often recurrence. Besides, there is no therapeutic target for this type of breast cancer because tumor cells do not have receptors for the targeted therapy (Chen et al, 2009) .

The degree of differentiation is one of the well-known prognosis parameters of its utilization. Assessment of the widely accepted degree of differentiation in breast carcinoma is a system based on Nottingham combination criteria (Elston-Ellis modification of the Scarff-Bloom-Richardson scaling system), which consists of tubular structure formation, core pleomorphism and mitotic number. The degree of differentiation is grouped into three categories: differentiation degree is good (degree 1), degree of moderate differentiation (degree 2) and degree of bad differentiation (degree 3). The worse the degree of differentiation, indicating tumor cells have an increasingly different ability with the original cell and growing faster. (Colditz et al, 2012; Lester, 2015).

Vascular invasion is defined as penetration of tumor cells into the lumen of the arteries or veins. Assessment of these parameters is particularly important in patients with metastasis to negative lymph nodes, as they can estimate the risk of metastasis to the lymph nodes. Vascular invasion is found in 5 - 50% of cases of breast cancer (Rosen, 2009; Colditz, 2012).

Under normal circumstances or homeostasis, tissue growth is determined by the rate of proliferation, differentiation and apoptosis. If the body experiences a disorder such as cancer then the rate of proliferation, and differentiation will be increased. Cell proliferation is cell division and cell growth, which is governed by the cell cycle. Under normal circumstances the cell cycle will be well controlled but in tumors or malignancies, the cell cycle will be dysregulated by cyclin and CDK (Cycline Dependent Kinase) activity, making it easier for cells to proliferate. Cell proliferation activity can be detected using Ki -67 immunohistochemistry that will express each phase in the cell cycle Kumar et al, 2015) Based on the Ki-67 expression, the recommendation of the St.Gallen Consencus Conference states that this expression is divided into low / low expressions and high expressions. This rate of proliferation is used as an indicator for adjuvant therapy (Esposito et al, 2015).

Based on the above description, the authors are interested to examine how the relevance of histopathologic characteristics (differentiation and lymphovascular invasion) with a marker of Ki-67 cell proliferation in triple-negative breast cancer.

MATERIALS AND METHOD

A total of 19 cases of invasive breast carcinoma which is known to include the triple-negative type of ER, PR, Her-2 / neu examination results, treated in the Surgical Department of Dr. M.Jamil Padang in the period collected from January 1, 2010 until December 31, 2013. Ki-67 expression has also been checked along with ER, PR, Her-2 / neu examination. The acquired Ki-67 expression is then grouped into low and high expression based on St. Gallen 2015 where ≤10% to 29% of low expression -sed; and> 30% high expression. Block paraffin and slaid breast cancer collected back then conducted a review to obtain data degrees of differentiation and lymphovascular invasion. The histopathologic characteristics assessed were degrees of differentiation and lymphovascular invasion. The degree of differentiation is determined by the combination of Nottingham criteria, consisting of degrees 1,2 and 3, then grouped again into low degrees (degrees 1-2) and high (degree 3). Lymphovascular invasion (LVI) is assessed by the presence / absence of tumor cells in the wall and / or the vascular lumen (positive / negative).

RESULT AND DISCUSSION

Retrospective studies of cases of triple-negative molecular subtype breast cancer were treated in the RSUP Surgery section. Dr. M. Djamil Padang from January 1, 2010 until December 31, 2013. During this period 19 cases of triple negative breast cancer from 66 cases of breast cancer (28%), which also conducted a Ki-67 examination.

Table 1. Distribution of degree of differentiation, lymphovascular invasion (LVI) and Ki-67 expression of triple negative breast cancer.

Characteristics	n	%
Degree of differentiation		
• Low degree	9	47,4
• High degree	10	52,6
LVI		
• Positive	13	68,4
• Negative	6	31,6
Ki-67 expression		
• Low to moderate	18	94,7
• High	1	5,3
Total	19	

Table 1 shows that the high degree of differentiation (degree 3) and positive LVI were more common in triple-negative breast cancer (52.6% and 68.4%), but high Ki-67 expression was found in only 1 case (5.3%).

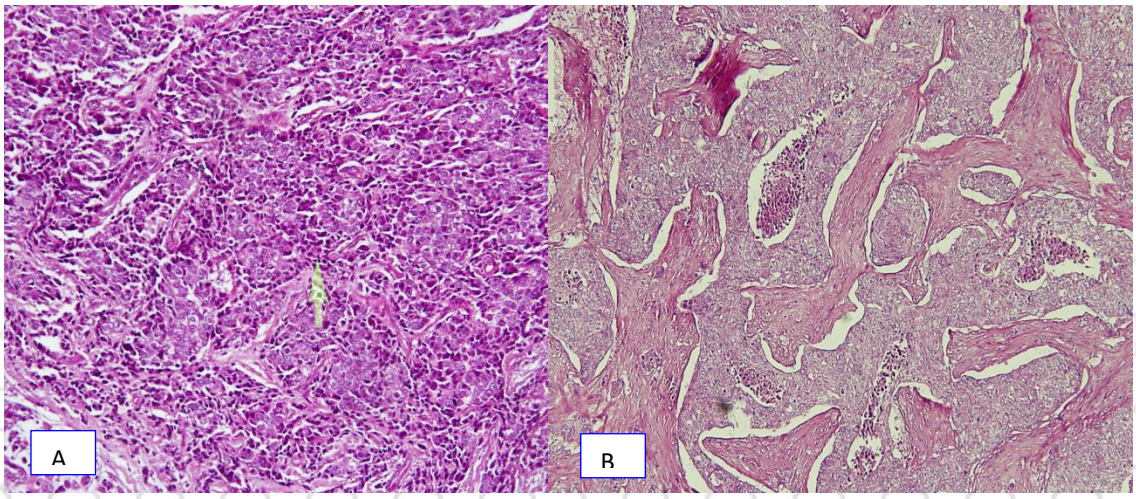

Figure 1 shows microscopic images of breast cancer with degree of differentiation; degrees 3 / high (A) and 2 / low (B). (HE x 400 and HE x 200).

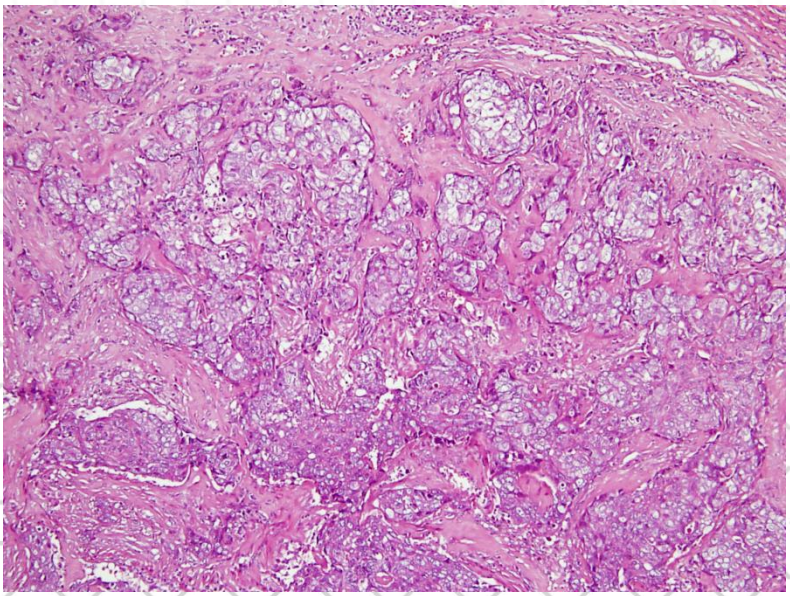

Figure 2. Triple-negative breast cancer with positive LVI. HE x 200.

Table 2. Relationship of Ki-67 expression with degree of differentiation

Ki-67 expression	Degree of differentiation		Total
	Low degree	High degree	
• Low to moderate	9	9	18
• High	0	1	1
Total	9	10	
p-value = 0,053 (α = 0,95)			

Table 2 shows that high Ki-67 expression was obtained in 1 case of triple negative breast cancer with high degree of differentiation. The statistical analysis did not show a significant relationship between the Ki-67 expression and the degree of differentiation.

Table 3. The relationship between Ki-67 expression and LVI

Ki-67 expression	LVI		Total
	Positve	Negatve	
• Low to moderate	12	6	18
• High	1	0	1
Total	13	6	
p-value = 2,579 (α = 0,95)			

From table 3 it was shown that high Ki-67 expression was obtained in 1 case of triple negative breast cancer with positive LVI. Statistical analysis did not show a significant relationship between the expression of Ki-67 and LVI.

During the period January 1, 2010 until December 31, 2013 found 66 cases of breast cancer in the department of surgery RSUP. Dr. M. Djamil Padang, where 19 cases (28%) were triple-negative molecular subtypes. This figure is not much different from the case in FKUI / RSCM which is 30% but higher than the number obtained by Su et al (2011) in China which is 12.9% of cases. Breast cancer in Indonesia ranks second after cervical cancer but ranks first in West Sumatra. More than 50% of breast cancer patients in Indonesia come at an advanced stage, with a low survival (BRK IAPI 2006; BRK IAPI 2009; Hardjolukito, 2010; Su Y et al, 2011). When viewed from a molecular subtype in which the triple negative number is large enough then this is likely to be one of the causes of low survival rates. Further research is needed on these low rates of survival rates and predisposing factors for triple-negative breast cancer in Indonesian women. Boyle P (2012) in his study found that triple negative breast cancer is more common in black, young and obese women.

Table 1 shows that high degrees of differentiation (grade 3) and positive LVI are more common in triple-negative breast cancer (52.6% and 68.4%). This is in accordance with research Kosasih et al (2011) in Sanglah Hospital, Bali who found 59.1% high-grade breast cancer. Similarly, in accordance with research by Rahniayu et al (2011) in RSUD Dr. Soetomo Surabaya who found the majority (64.7%) of triple negative breast cancer with high degree of differentiation / 3. Higher degrees of differentiation are associated with more aggressive tumor properties and a worse prognosis. The higher degree of differentiation is associated with higher proliferation as well.

Lymphovascular invasion (LVI) is a very important stage in the process of metastasis. At the St. Gallen 2005, LVI was included as a prognostic factor in breast cancer without metastasis to lymph nodes (KGB), wherein mortality of breast cancer patients without KGB metastasis but with LVI (+) higher than LVI (-). LVI is also associated with other prognostic factors such as tumor size and degree of differentiation. In this study found 68.4% of cases with LVI (+). From the literature also mentioned that triple negative breast cancer has a more aggressive clinical and pathological features than other molecular subtypes, although more response to chemotherapy. This is due to the short duration of disease free-survival and more likely to

metastasize (Kosasih et al, 2011; Rahniayu et al, 2010; Ismail-Khan et al, 2010; Mohammed ZMA et al, 2011).

Ki-67 is a protein expressed in the cell nucleus during the cell cycle. The higher the Ki-67 expression, the more cells (tumors) that proliferate. Therefore Ki-67 is now widely used as a marker of proliferative activity. Ki-67 expression was used as a predictor for the response of hormonal therapy and chemotherapy. Some researchers found a correlation between Ki-67 expression with time of free-survival disease and survival rates and a higher recurrence risk in tumors with high Ki-67 expression. Various methods used to assess the expression of Ki-67 include immunohistochemistry, PCR, tissue micro array (Beresford et al, 2006; NordiQC). . Various methods were also applied to determine the cutpoint and standardization of Ki-67 expression assessment using immunohistochemical methods, including Allred scoring (Allred, 1998). St Gallen (2015) set a cutpoint for breast cancer that is ≤10% to 29% low to moderate expression; and> 30% high expression. Widodo et al (2013) found a significant association between lymphangiogenesis with tumor size, degree of differentiation, KGB status, LVI, p53 and Ki-67 expression. From table 2 and 3 it was seen that Ki- 67 high was obtained in 1 case of triple negative breast cancer with high degree of differentiation and positive LVI. Statistical analysis did not show a significant relationship between the Ki-67 expression and the degree of differentiation and between the Ki-67 expression and LVI. These results are different from those found by other researchers where high Ki-67 expression was found in 74.6% of breast cancer cases (Ermiah et al, 2012). Many technical factors may affect the results of staining and Ki-67 assessment by immunohistochemical methods. According to the recommendations of the Breast Cancer Working Group, the tissue samples used from the core biopsy and the whole tumor should be fixed with a formalin buffer, in staining with positive control and negative control, using a microwave for retrieval antigen, the antibody suggested is MIB1, is nuclear staining and the assessment is performed at least on 500 invasive tumor cells Dowsett et al, 2011). Immunohistochemical staining is also highly determined by tissue processing when creating paraffin blocks. Although it is recommended, many hospitals have not used formalin buffer as a fixative solution. In addition there are not many laboratory Pathology Anatomy that has been standardized and do Quality Assurance so that examination results, especially immunohistochemistry also vary.

CONCLUSION

High Ki-67 expression is found only in 1 case, whereas the highest degree of differentiation (degree 3) and positive LVI are more common in triple-negative breast cancer. This suggests that histopathologically, triple negative breast cancer is more progressive. Technical considerations should be considered in staining and assessing Ki-67 expression. There was no significant association between the Ki-67 expression and the degree of differentiation and lymphovascular invasion of triple-negative breast cancer. It is recommended to do further research with a larger sample size, a standardized assessment of Ki-67 expression and be associated with survival of the patient.

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Antiproliferation Activity of *Gelidium latifolium* Ethanol Extract in Human Caco-2 Cells

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Abstract

Seaweed is a source of various bioactive compounds due to the number of secondary metabolites synthesized. However, information on biological activities for bio-prospecting is still very limited. This study evaluated the antiproliferative activity of the algal *Gelidium latifolium* ethanol extract (GLE). Determination of bioactive compounds contained in crude ethanol extract GLE was carried out by GC-MS analysis. Meanwhile, to determine the effect of antioxidant extract DPPH assay was conducted. Antiproliferative activity of *G. latifolium* on Caco2 cells was carried out by calculation of total cell density by trypan blue exclusion assay after 72h. Together with this observation the effects of GLE on Caco2 cell morphology was also analyzed in phase-contrast microscope. From the GC-MS chromatogram analysis, high concentration of palmitate acid was detected compared to other existing compounds in GLE. Antioxidant test results showed IC_{50} 167 µg/mL. Crude ethanol extract GLE showed a significant antiproliferative effect with IC_{50} 138 µg / mL. This research is expected to be the initial information and reference for further research in the development of macroalgae, especially *G. latifolium* in the field of seaweed-based cancer treatment.

Keywords : Caco-2, macroalgae, *Gelidium latifolium*, antiproliferative

INTRODUCTION

Cancer is one of the most dangerous and deadly diseases in the world. According to the World Health Organization (WHO) there are 8.2 million cases of cancer deaths from 14.1 million cases of cancer worldwide (WHO, 2012). Nearly 70% of these deaths occur in low- and middle-income countries including developing countries including Indonesia. The Ministry of Health reported the prevalence of cancer in Indonesia in 2013 was 1.4% or estimated at 347,792 people and predicted to continue to increase in the future (Depkes RI, 2015).

Cancer treatment generally combines surgery and radiation with treatment chemotherapy (Sukmarianti *et al.*, 2013). Which are expansive and unaffordable for most of the people in Indonesia. Furthermore, chemotherapeutic drugs are evidenced to provide side effects such as nausea, vomiting, hair loss, reduced levels of white blood cells in the blood, and irritation of the urinary contents, as well as other side effects (Abeloff *et al.*, 2004; Sumarianti *et al.*, 2013). This encourages scientists to explore anticancer bioactive compounds from natural sources that are expected to provide lower side effects and more affordable prices.

Seaweeds are large and diverse groups of that are rich in active metabolites and a source of novel ingredients for functional foods. Seaweeds are also considered as source of bioactive compounds as they are able to produce a great variety of secondary metabolites, characterized by a wide range of biological activities such as anti-microbial (Lutfiyanti *et al.*, 2012; Elsie & Dhanarajan, 2010; Metidji *et al.*, 2015; Abou-Elella & Mahgoub, 2015), anti-inflammatory (Abou-Elella & Mahgoub, 2015); as well as anti-tumoral activities (Metidji *et al.*, 2015; Abou-Elella & Mahgoub, 2015; Alghazeer *et al.*, 2016).

Gelidium latifolium belongs to the genus of red algae which can be found in intertidal to subtidal regions. *Gelidium* sp has a high abundance in Indonesia but has not been utilized and cultivated by the community even though it has been reported to have several benefits as food and medicine (Atmaja & Sulistijo, 1998; Sunarpi *et al.*, 2005; Sukiman *et al.*, 2014).

This study aims to evaluate pharmaceutical properties of *G. latifolium* for potential medicinal applications. Pharmaceutical properties of *G. latifolium* were determined by investigation of antiproliferative activity of red algae *G. latifolium* crude extract on the growth of Caco-2 cancer cells in vitro model. The Caco-2 cell line, which exhibits a well-differentiated brush border on medical surface and tight junctions, and expresses typical small-intestinal microvillus hydrolases and nutrient transporters, has proven to be the most popular in vitro model (a) to rapidly (b) to elucidate pathways of drug transport (eg, passive versus carrier mediated), (c) to assess the membrane permeability formulation strategies designed to enhance, (d) to determine the optimal physicochemical characteristics for passive diffusion of drugs, and (e) to effect the effects of drugs on this biological barrier (Maunier et al., 1995). This research is expected to provide additional information and contribute to the development of seaweed-based cancer drugs.

MATERIALS AND METHOD

A. Seaweed Collection:

Seaweed *Gelidium latifolium* were collected at low tide and during the spring tide by hand-picking in the period of January from Lendang Luar, Malacca Village, East Lombok, West Nusa Tenggara, Indonesia (116,0360 ºS - 8,4627 ºE). The algae were cleaned, washed in distilled water, then dried at room temperature and crushed until a fine powder was obtained.

B. Chemicals:

Chemical used in this study were 96% ethanol, 70% ethanol, phosphate buffer saline (PBS), FBS (Fetal bovine serum), DMSO, Caco2 cell line, ascorbic acid, aquadest, aquabidest, DPPH 0.15 mM, 1% hepes, absolute ethanol, trypsin-EDTA, $NaHCO_{3(l)}$, trypan blue, Dullbecco's modified Eagle's medium (DMEM, supplemented with 1% penicillin-streptomycin, 10% FBS, and 1% n-glutamine), $N_{2(l)}$.

C. Preparation of extracts:

The powder of dried algae was extracted in ethanol described by Rohmah (2016). Dried *S. cristaefolium* were mashed in order to get the same particle sizes and extracted with 96% ethanol (1:5 m/v). These extract then filtrated and evaporated to dryness at 45-64 ºC. The extract rendement were measured using the following formula:

$$\text{Rendement} = \frac{\text{sample weight}}{\text{extract weight}} \times 100\%$$

The resulting extract were concentrated to dryness in a rotary evaporator under reduced preassure (at 45˚C) until a crude extract was obtained and was conserved at 4ºC.

D. Phytochemicals Chemistry

Powdered samples of each investigated algae of *Gelidium latifolium* was subjected to phytochemical screening as published by Riyanto (2013) with gas chromatography-mass spectrometry (GC-MS) to investigate their phytochemical constituents. Five miligram of SC crude extract was diluted to final concentration of 1000 µl in absolute ethanol and analyzed using GC-MS at Laboratory of Analytical Chemistry, Faculty of Mathematics and Natural Sciences, University of Mataram.

E. Antioxidant Assay

The antioxidant activity of *Gelidium latifolium was* determined using DPPH free radical scavenging assay as described by Suhaling (2010). All estracts were dissolved in 97% ethanol and a series of concentration dependent dilutions were made (50, 100, 150, and 200 µg / mL). Standard reagents were utilized for comparational for all antioxidant assays.

The percentage (%) inhibition of the DPPH radical was calculated by using following equation:

$\%\ AA = [(A_0-A_1)/A_0]x100\%$

A_0 is the absorbance of the control and A_1 is the absorbance of the samples at different concentrations. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of scavenging effect percentage against extract concentration.

F. Cell Culture

Human colon cancer cell line (Caco-2) cells were routinely cultivated in Dulbecco's Modified Eagle's Medium (DMEM, Wako) supplemented with 10% fetal bovine serum (FBS) at 37ºC in a 5% CO_2 humidified incubator. For all experiments, Caco2 cells were plated in 35 mm culture dishes, incubated overnight, and incubated in DMEM supplemented with specific concentrations of macroalgae extracts (50, 100, 150, and 200 µg/mL). Cell images were obtained from phase-contrast microscopy by BZ-9000 microscope (Keyence, Osaka, Japan).

G. Cytotoxicity Assay

Potential cytotoxic compounds are tested in the Bioscience and Biotechnology Research Center, West Nusa Tenggara, Indonesia. Antiproliferative effects of *G. latifolium* was determined by total cell count with trypan blue exclusion assay (Prasedya *et al.*, 2016). Cells were plated in 35 mm cell dishes for initial seeding. After an additional 24 h, various concentrations of crude G. *latifolium* (50, 100, 150 and 200 µg / mL) were incubated for 72 hours at 37 ºC. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), the cells were incubated for 4 h at 37 ° C. The medium was removed and formazan was dissolved in DMSO. The effect of the extracts on the proliferation of cells was expressed as total cell count of Caco-2 cells treated after 72 hours. All experiments were performed at least twice in triplicate.

Morphological changes of cells untreated and treated with algae were performed by inverted microscope (Nikon Eclipse T*i*).

RESULT AND DISCUSSION

1. Determination of Plants and Extraction

Figure 1. Morphological appearance of *G.latifolium*. Scale: 24 mm.

Red macroalgae *Gelidium Latifolium* samples were collected from the waters of West Nusa Tenggara. The red color of the seaweed thallus is caused by the pigment phycoerythrin (Basuki, 2008). Many could be found in tropical seas, especially in intertidal and subtidal regions. *G.*

latifolium has a length of approximately 20 cm and a width of 1.5 mm. The reproductive organs are macroscopic. The cystocarp has a small hole (osteolo) on both sides of the thallus, tetraspora divides krusiat or tetrahedral. Krusiat is one spore arrangement (Aslan, 1991).

The results of ethanol crude extraction *Gelidium latifolium* has a yielding weight of 5.3337% with the form of extract in the form of a rather liquid paste and blackish green (Table 1).

T**able 1.** Rendement (%) of macroalgae *G. latifolium* subjected to ethanol solvent extraction

Weight Dry	Weight Macerate	Weight of Extract	Rendemen t	Color Extract	Form Extract
833.4 grams	539.4 grams	28.77 grams	5.3337%	Green Blackish	Pasta rather liquid

2. Phytochemical Analysis of GC-MS

The bioactive compounds present in crude extracts obtained from GLE-EtOH are shown in Figure 2. Their identification and characterization was analyzed with GC-MS and found to have bioactive compositions. Results of GC-MS analysis shown that GLE crude ethanol extract consist of 40 different bioactive compounds. The retention time, molecular formula and the amount of these bioactive compounds were also presented (Table 2). Based on abundance, top there major compounds presenst present in GLE crude extract were palmitic acid (53.74%), isocopent (8.20%), and linoleic acid (8.17%).

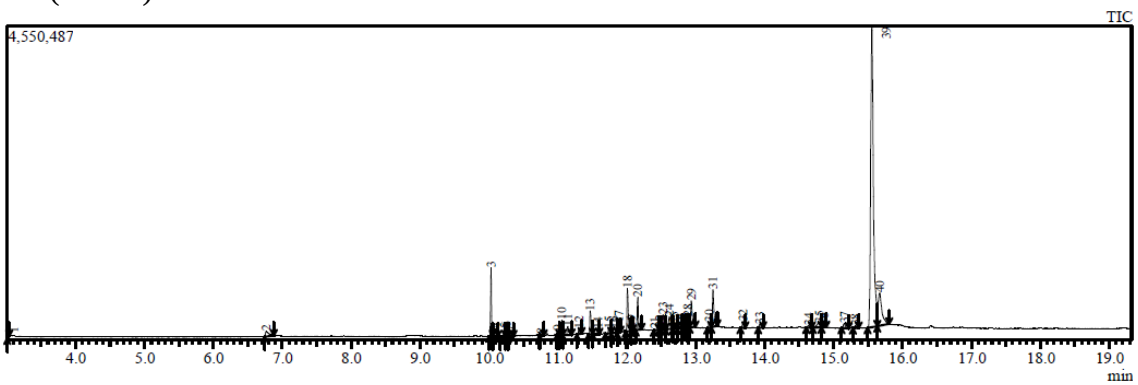

Figure 2. GC-MS analysis of G.latifolium crude ethanol extracts.

Palmitic acid is a common saturated fatty acids found in animals and plants, a saturated fatty acid found in fats and waxes including olive oil, palm oil, and body lipids. Hexadecanoic acid is a group of palmitic acid oracid hexadecanoic and has the ability to induce apoptosis in testing against HepG2 cancer cells (Zhang *et al.*, 2004). Palmitic acid is a saturated fatty acid that is commonly found in many kind of terrestrial plant and brown macroalgae as well. High concentration of palmitic acid in an organic materials provide better protections from UVR and pollutant (Alvarez & Rodriguez,

2000). Palmitic acid is a unique chemichal compound with double bond structure that absorbs UVR (Grollier & Plessis, 1998). It also has stable structures in chemical and thermal reactions and it's less toxic (Rosen, 2003). Linoleic acid is a doubly unsaturated fatty acid, also known as an omega-6 fatty acid, occurring widely in plant glycosides. It is used in the biosynthesis of prostaglandins and cell membranes.

Table 2. Important Compounds of Crude Extracts *Gelidium latifolium*

Compound name	Retention Time	Area (%)	Molecular Form	Molecular weight
Aristolene	10.030	3.95	$C_{15}H_{24}$	204.357 g/mol
Octadecane	11.076	1.05	$C_{18}H_{38}$	254.502 g/mol
Myristic acid	11.490	2.55	$C_{14}H_{28}O_2$	228.376 g/mol
Linoleic acid	12.032	8.17	$C_{21}H_{44}$	280.452 g/mol
Stearic acid	12.182	2.71	$C_{18}H_{36}O_2$	284.484 g/mol
Phytol	12.556	1.62	$C_{20}H_{40}O$	296.539 g/mol
Oleic acid	12.963	3.29	$C_{18}H_{32}O_2$	282.468 g/mol
Heneicosane	13.284	4.09	$C_{16}H_{32}O_2$	296.583 g/mol
Palmitic acid	15.557	53.74	$C_{16}H_{32}O_2$	256.430 g/mol
Icospent	15.669	8.20	$C_{20}H_{30}O_2$	302.458 g/mol

3. Antioxidant Test

Antioxidant testing in this study was used as additional data to test whether there was antioxidant activity from the red algae ethanolic extract of GLE The method used in the determination of antioxidants is DPPH (*1,1-diphenyl-2-picrylhidrazyl*) DPPH method is an effective and fast colorimetric method for estimating antiradical / antioxidant activity. DPPH radical (*1,1-diphenyl-2-picrylhidrazyl*) is an organic compound containing nitrogen unstable with strong absorbance in the wavelength range 511 nm and dark purple. After reacting with the antioxidant compound, the DPPH will be reduced and the color will turn yellow. These changes can be measured by a spectrophotometer, and plotted against concentration (Reynertson, 2007). The decrease in color intensity that occurs due to reduced conjugated double bonds in DDPH. This can occur if there is an electron capture by an antioxidant, causing an absence of electron chance to resonate (Pratimasari, 2009). The presence of an antioxidant which can contribute electrons to DPPH, produces a yellow color which is a specific characteristic of DPPH radical reactions (Vaya and Aviram, 2001). Free radical scavengers cause electrons to pair up which then causes color loss that is proportional to the number of electrons taken (Sunarni, 2005). Results of antioxidant assay with DPPH shown in Table 3.

Table 3. DPPH analysis of SC Crude Ethanol Extract Compared to Ascorbic Acid

Sample Name	Concentrations (µg/ml)	Absorbance at 511 nm	%AA	IC50 (µg/ml)
G. latifolium Crude EtOh Extract	50	0.104	30.837	167
	100	0.137	9.0308	
	150	0.148	2.202	
	200	0.117	22.467	
Ascorbic Acid	30	0.022	85.24229	89.5
	100	0.019	87.6652	
	150	0.015	89.86784	

GLE crude ethanol extract antioxidant activity was medium compared to ascorbic acid. GLU crude ethanol extract able absorbed 2-30% DPPH free radical compound at 50 to 200 µg/mL estract concentrations compared to ascorbic acid that absorbed 85-89% DPPH free radical compounds in same concentrations range. The scavenging effect with the IC_{50} values of 167 µg/mL it has more than control (ascorbic acid) IC_{50} value with 89.5 167 µg/mL. an antioxidant property are affective when it has maximum IC_{50} value of 200 ppm. GLE crude ethanol extract have less than 200 ppm IC_{50} value which mean it has good antioxidant activity.

Several researches have been reported that methanol crude extract of *Geldium latifolium* was low antioxidant with 300 ppm IC_{50} (Alghazeer et al., 2016). The low antioxidant activity is probably caused by a variety of factors, including because the extraction method used may not be enough to attract the antioxidant chemical components in *G. latifolium*. In addition, because vitamin C is a pure compound while the ethanol crude extract *G. latifolium* is still a mixed compound and the antioxidant content of the compound is unknown, the presence of non-antioxidant compounds may affect the antioxidant activity of the red algae extract of *G. latifolium* it self.
Seaweed is included in the types of marine plants that are most widely used as a source of medicine by humans because they contain various bioactive compounds that have benefits in the field of medicine. One of them is as an antioxidant. For this reason, this study conducted antioxidant testing because antioxidants can maintain the stability of cell metabolism so as to prevent cancer.

4. Cytotoxicity Test

Cytotoxicity test is antest *in vitro* that uses culture cells which are used to detect the presence of antineoplasmatic activity of a compound. The use of cytotoxicity tests on a cell is one way of determining *in vitro* to get cytotoxic drugs. This system is a qualitative test by determining cell death. The end of the cytotoxic test can provide

information on drug concentration that still allows cells to survive (Doyle & Griffiths, 2000).

Cytotoxic tests are used to predict the presence of new cytotoxic drugs from natural ingredients that have the potential as anti-cancer agents. The basis of this experiment, among others, is that the system of determining biological activity will produce a response dose curve and response criteria which should show a straight relationship with the number of cells. Information obtained from the curve should be related to the effect in vivo of the same cytotoxic drug (Padmi, 2008).

This anticancer test aims to determine the antiproliferative activity of red algae extracts of *Gelidium latifolium* on the growth of cancer cells Caco-2 in various concentrations of 50, 100, 150, and 200 µg / mL (Figure 3). This anticancer activity test was carried out *in vitro* using colon cancer cells Caco-2 by method direct counting. This test in vitro uses cell line which gives advantages compared to testing in vivo, ie the test material used is less and the testing time is relatively short.

Figure 3, shows the percentage change in the growth inhibition of cancer cells treated with GLE crude ethanol extract. The algae extract tested displayed a substantial inhibitory effect (85%) at 200 µg / mL, compared with control (25-30%). Antiproliferative effects of red algae *G. latifolium* were investigated with total count via trypan blue exclusion assay. Cytotoxicity activity of *G. latifolium* increased in adose dependent manner (Figure 3). After 72h, the IC50 value of GLE treated cells (138.76 µg / mL) was significantly higher compared to control.

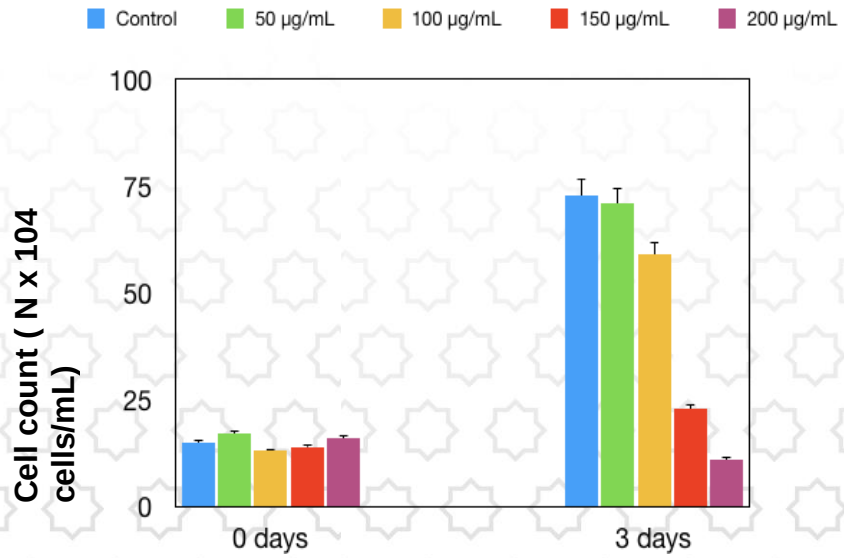

Figure 3. Percentage growth inhibition of cancer cells treated with GLE crude ethanol extract.

The latest phytochemical studies confirm the presence of bioactive compounds such as saponins, flavonoids, tannins and polyphenol components in the most tested algae (Zakaria *et al.*, 2011; Alghazeer *et al.*, 2014; Kharthick *et al.*, 2014). Therefore, the cytotoxic effect of algae, through inhibition of Caco2 cell proliferation is probably related to the content of these compounds, especially polyphenols and flavonoids (Salucci *et al.*, 2002). For example, quercetin shows antioxidant activity that is believed to have a cytoprotective role in oxidative stress (Du *et al.*, 2010). In addition, the presence of 2.3-double bonds in flavonoid molecules correlates with mitochondrial damage and cancer cell death (Plochman *et al.*, 2007).

Cytotoxicity is an activity that is consistent with anticancer activity, the major advantage of cytotoxicity assays is that all potential mechanisms of cellular ploriferation can be monitored simultaneously (Alghazeer et al., 2016). Gelidium has several compounds that have cytotoxic activities such as triterpenoids, steroids, and palmitate acid (Lutfiyanti, 2010). According to Zakaria *et al* (2011) that some compounds that are thought to be able to inhibit cell growth are from flavonoids, tannins, saponins and triterpenoids. The mechanism of action of the group of flavonoid compounds is to modulate the cell cycle holding in phase G1 to phase S. The mechanism of action of saponins and triterpenoids by damaging the permeability of mitochondrial membranes in cells or causing cells to experience necrosis and death.

Some previous studies stated that some types of algae can inhibit cell growth in several ways such as dose and time levels. The results of this study are in accordance with research conducted by Alghazeer *et al*., (2016) which conducted antiproliferation tests of several algae extracts in cells Caco-2. in vitro including *Gelidium latifolium*. The ability of ethanol crude extract *G. latifolium* to induce cell death was estimated by analyzing its effect on cell morphology. The observation of Caco2 cells under a phase contrast microscope showed that after 72 hours of threatment at a concentration of 200 µg/mL has an inhibitory ability of 85% and the highest percentage of cell population occurs in the G0 phase and the lowest in the Sub G phase [(9)]. However, in this study, at a concentration of 200 µg/mL cells Caco-2 could no longer live and develop but only floated on the surface and could not stick to the base of the dish.

Microscopic observation in Caco2 cells showed altered morphology after 72h of *G. latifolium* treatment (Figure 4). Caco2 cells were seen to shrink in size and change into a circular shape. Furthermore, losing adherent ability which implies the cells are possibly undergoing apoptosis. Meanwhile, untreated Caco2 cells exhibit an oval-shaped like needles shape that stick together with other cells around them and adherent to the base of the culture dish

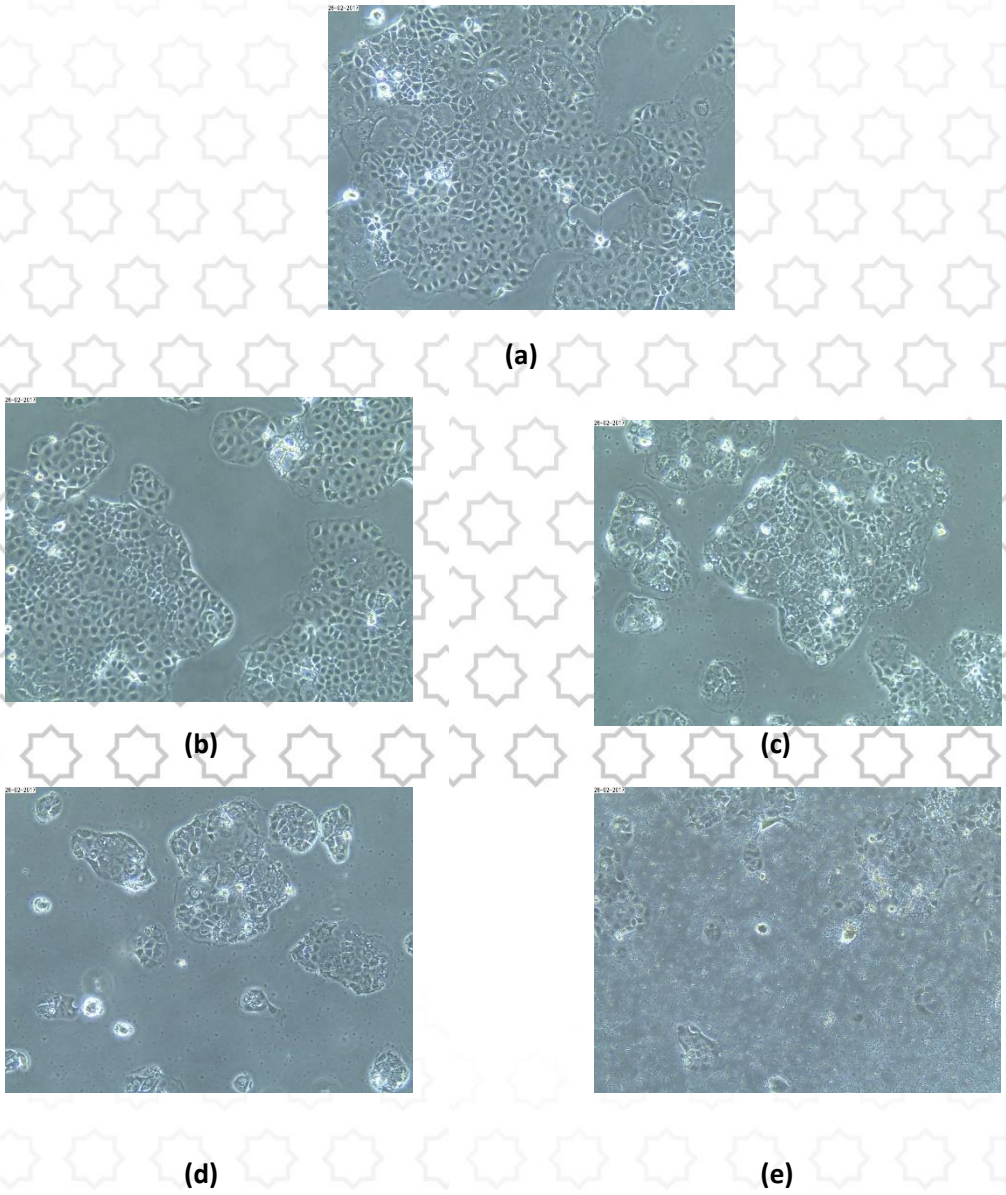

Figure 4. Microscopy analysis of Caco2 cells treated with different concentrations of *G.latifolium* extracts for 72h. (a) Control; (b) 50 µg/mL; (c) 100 µg/mL; (d) 150 µg/mL; (e) 200 µg/mL)

The ability of algal extract to induce cell death was estimated by analyzing its effect on cell morphology. Observation of Caco2 cells under a contrast phase microscope showed that after 72 hours of treatment with 200 µg / mL extract, detectable changes were found, including changes in cell morphology, cell shrinkage and membrane blebbing, characteristic features of apoptotic cell death.

It is very important to maintain homeostasis between cell proliferation and cell death in normal mammalian tissue; therefore, the process by which cell proliferation rates exceed

cell loss in tumor cells can be suppressed or disturbed (Minko *et al.*, 2003). Previous research reported that some algae extracts inhibit cell growth in a dose-dependent and time-dependent manner, by blocking the development of cells and by promoting apoptosis in the HCT-116 colon cancer cell line (Palozza *et al.*, 2009).

Many chemotherapy agents are found to be selectively toxic to tumor cells because they increase oxidant stress and increase cells that are already stressed out of their limits (Moungjaroen *et al*., 2006); In contrast, the anticancer activity of plant compounds can be associated with high affinity for targets, less entropy loss when they bind to proteins and their bioavailability. In addition, plant compounds are considered to have conformational flexibility in aqueous and lipophilic environments (McCullagh, 2008) and can act as a good alternative anti-cancer agent.

There is a growing need for the development and or discovery of bioactive compounds that are very potential from natural sources because of resistance to chemical drugs.

CONCLUSION

In conclusion, algae *G. latifolium* are avaluable source of natural antioxidant compounds as thir crude extracts exibits antioxidant activity. The results of antioxidant activity and antiproliferative activity of Caco2 cells by crude ethanol extract of GLE in a dose of 50, 100, 150 and 200 µg/mL and showed a significant antiproliferative effect with IC_{50} 138 µg/mL. GLE crude ethanol extract showed a medium antioxidant effect with IC_{50} 167 µg/mL and have potential antioxidant activity. However, further investigation is needed to assess the molecular mechanisms oh the potential anticancer activities of GLE extract as well as to identify. Bioactive compounds found in GLE await a major breakthrough for a variety of food/medical application as they have the potential for application as natural antioxidant in different food/pharmaceutical industry.

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Ethnopharmacy Study of Tengger Tribe in Ledokombo and Pandansari Village, East Java

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Abstract

Ledokombo and Pandansari village, Sumber Sub-District, Probolinggo District of East Java are several of Tengger tribe's residence area that still preserve their ancestral heritage culture, such as traditional medicine which is inherited verbally. The absence of medication documentation and the influence of modern culture caused decreasing of traditional medicine utilization. The study investigated the use of traditional medicine by the people of Tengger tribe in Ledokombo and Pandansari village as an effort to preserve the knowledge of traditional medicine. The method used in this research was semi-structured interview by using open-ended question with snowball and purposive sampling technique. The study was conducted to 29 informants who knew and/or used traditional medicine. The results showed that there were 15 types of diseases suffered by Tengger tribe, and 38 species of medicinal plants in 67 traditional recipes. The most widely used parts of the plants was leaves (37.50%). The common method to prepare the recipes was raw crushing in water (35.37%). Meanwhile, peroral administration by drinking was the most common method in consuming the traditional medicines (40.58%). The results showed that kunir (*Curcuma longa*) (0.660), jahe (*Zingiber officinale*) (0.485) and tepung otot (*Stellaria media*) (0.448) had the highest UV (Use Value). Moreover, thrush (1), skin disease (1), dizziness (1), sprain (1), cough (1) and diarrhea (0.8) had the highest value of ICF (Informant Consensus Factor).

Keywords : Tengger tribe, medicinal plants, UV, ICF

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INTRODUCTION

Recently, the synthetic medicine has become a trend among the society. The trend is shown by the existence of drugstore, pharmacy industry, and national pharmacy market. The high use of the synthetic medicine among the society was revealed by Natu (2015) research which showed that people of Pentadu subdistrict Paguat district Pohuwato regency 85 (88.4%) chose the synthetic medicine and 11 people (11.6%) chose the traditional medicine.

The high use of synthetic medicine does not indicated that it has no side effect. The report derived from the Health Workers and Pharmaceutical Industry from 2010 until 2014 showed that medicine side effect had increase significantly (BPOM, 2015). According to Marianne et al. (2011), the side effects of the medicine and the high medical cost for a long-term medication often caused the failure of the therapy by using synthetic medicine. To solve this problem, alternative therapy by using natural materials is needed.

The use of the traditional medicine to protect the health and to cure diseases had been developed (Efremila et al., 2015). The traditional medicine is commonly used as a self-alternative medication by the society in which it is a knowledge heritage from the ancestor both oral and written, based on the their experience (Riswan and Andayaningsih, 2008). The medication tradition that had been inherited orally has been not documented and clinically tested. Therefore, it caused the lack use of traditional medicine from time to time. The fact encouraged the researcher to conduct an ethnopharmacy research to protect the traditional medication knowledge from modernization.

Ethnopharmacy is an interdisciplinary knowledge relates to the pharmaceutics and certain culture terms which characterized the medicine usage in a number of human groups (Pieroni et al., 2002). The result of ethnopharmacy research is a traditional medication in a certain ethnic that can be compared to the modern medication result in a better understanding (Syifa et al., 2011). Ethnopharmacy approach is conduced by using several methods such as interview, plant documentation by using several digital camera and recording, herbarium, and field study of medicine plantation (Abel and Kofi, 2005 in Syifa et al's journal, 2012).

The Tengger tribe is one of many tribes in Indonesia which according to Sutarto (2006), still conserves on the tradition inherited by their ancestor. Ledokombo and Pandansari villages Sumber district Probolinggo regency are two of the Tengger tribe residential areas that still maintain their culture, including the traditional medication, yet it has not documented. Therefore, it is necessary to perform a research about the usage of the traditional medicine by Tengger tribe in Ledokombo and Pandansari villages Sumber district Probolinggo regency to conserve the traditional medication knowledge and to give a reference about drug discovery from natural materials.

MATERIALS AND METHOD

The data were obtained in September 2016 by using descriptive research through a semi-structured interview and an open-ended question. The interview was conducted by using key informant approach and snowball sampling, as well as purposive sampling techniques. The data analysis was carried out by using a quantitative approach. The data analysis used by the researcher was Informant Consensus Factor (ICF) and Use Value (UV).

RESULT AND DISCUSSION

The ethnopharmacy data were obtained from 29 informants aged between 42-96 years old. 15 types of diseases were chosen based on the ICF value which assumed as high by the researcher. From those diseases, 38 plant species and 67 receipts were collected as the traditional medicine by the society of Tengger tribe in Ledokombo and Pandansari villages Sumber district Probolinggo regency. Table 1 showed the local name, scientific name, family, UV (Use Value), part use, medicinal use, preparation, administration way and ICF (Informant Consensus Factor) value.

The plants part used, method of preparation and medicinal usage

The ethnopharmacy study of Tengger tribe in Ledokombo and Pandansari villages Sumber district Probolinggo regency revealed that there were some sources to obtain plants as medicinal materials, such as from the home yard and wild plants which grew around garden and forest. The plant's organ which were mostly used as the medicinal preparation in which commonly known to be identified by the informants were leaves (39%), rhizomes (18.06%), fruits (9.72%) (Figure 1). The leaves were used for photosynthesis accumulation which containing several organic contents, such as essential oils, phenols, calcium compounds, and chlorophylls as well as vitamins and minerals (Mahfudloh, 2011).

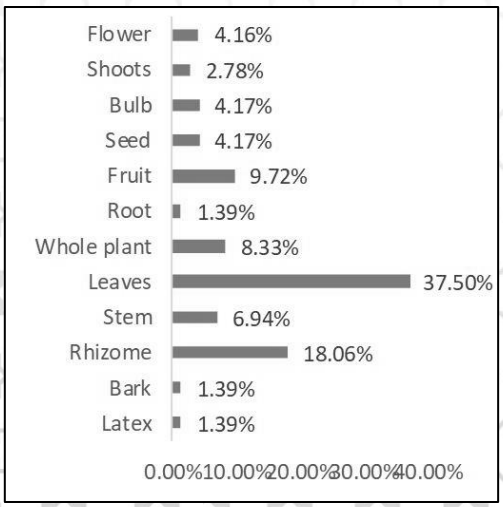

Figure 1. The plants parts used for preparing medicines

Whereas, the making process of traditional medicine was mostly by raw crushing in water (35.37%) and boiling (29.27%) (Figure 2). The medicine usage was mostly by drinking (40.58%), smearing (30.43%), sticking the herbal around the pain (18.84%), eating (5.80%), used for bathing (2.90%), and dropping (1.45%) as shown in Figure 3. In several cases, the different material were added in the process of the medicine preparation, such as sugar and salt to cover the bitter taste of medicinal plants. Futhermore, sugar and salt were added in preparation of temu ireng *(Curcuma Aeruginosa)* rhizome to cure itchy. Garlic, pepper, dringu *(Acorus calamus)*, nutmeg, green coconut, and palm sugar were used together as the medicinal herbs to reduce fever.

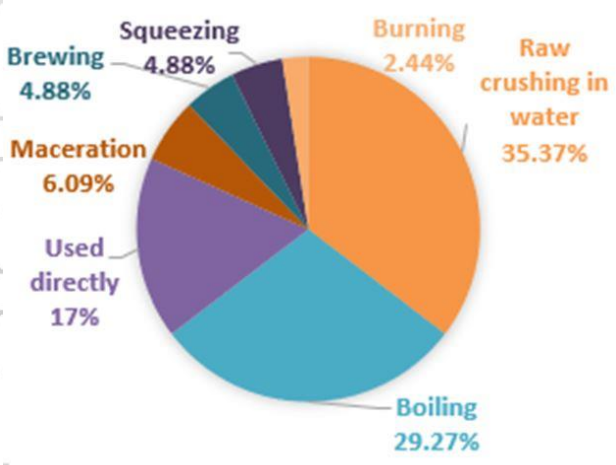

Figure 2. The method of traditional medicine preparation

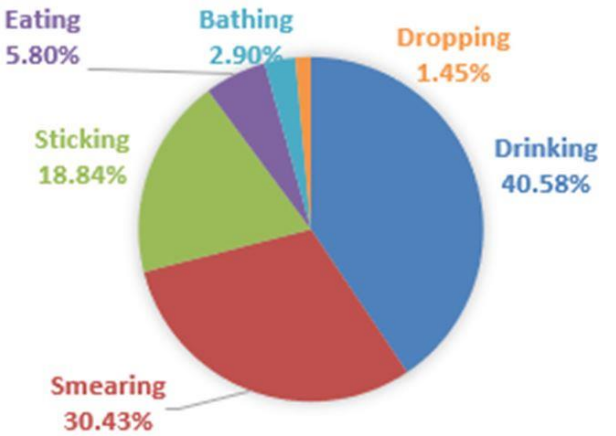

Figure 3. The plants medicinal usage

The Ethnopharmacy Quantitative Analysis

Traditional medicine information of the ethnopharmacy data was analysed qualitatively. Then, a quantitative analysis was carried out using the ICF and UV methods to determine diseases and medicinal ingredients that were considered important and efficacious in a particular tribe

(Gozzaneo et al., 2005; Almeida et al., 2006). In this study, there were several diseases that often suffered by the Tengger community. These results were based on high ICF values, namely thrush, skin disease, dizziness, sprain, cough, and diarrhea. The diseases with high ICF values (close to 1) indicated that those diseases often suffered the surrounding community. The majority of people who worked in the agricultural sector made it possible for many people to experience sprains. The majority of the Tengger tribe people also did not have a decent toilet. The lack of environmental sanitation facilities, especially fecal disposal or human excreta, can cause direct or indirect relationships between feces and humans, and the occurrence of transmission of Fecal Borne Diseases from the sufferers to healthy people as well as environmental pollution in general (Putranti and Sulistyorini, 2013). The poor living patterns were possible to cause the high rates of thrush, skin diseases, dizziness, and diarrhea suffered by the Tengger tribe.

Based on the high UV of kunir (*Curcuma longa*) (0.66), jahe (*Zingiber officinale*) (0.485), and tepung otot (*Stellaria media*) (0.448), it was known that those medicinal materials were considered important by the surrounding community. Therefore, it was necessary to perform futher testing related to drug discovery.

Table 1. List of medicinal plants used as traditional Tengger Tribe health care medicine

No	Local name	Scientific name	Family	UV	Part use	Medicinal use	Preparation	Administration way	ICF
1.	Jahe	*Zingiber officinale*	Zingiberaceae	0.485	Rhizome	Cough	Infusion	Internal	1.000
						Stomach ache	Infusion	Internal	0.167
2.	Kunir	*Curcuma longa*	Zingiberaceae	0.655	Rhizome	Cough	Infusion	Internal	1,000
						Itchy	Pounded	External	0.143
						Fever	Pounded	Internal	0.500
						Dizziness	Decoction	External	1.000
						Wound	Direct ingestion	External	0.500
						Stomach ache	Pounded	Internal	0.167
3.	Tepung otot	*Stellaria media*	Carophyllace-ae	0.448	Whole plant	Wound	Pounded	External	0.500
						Rheumatic pain	Pounded	Internal	0.545
						Sprains	Pounded	External	1.000
4.	Kecubung	*Datura metal*	Solanaceae	0.310	Flower	Eye pain	Direct application	External	0.556
5.	Dringu	*Acorus calamus*	Acoraceae	0.310	Rhizome	Convulsions	Pounded	External	0.667
6.	Merica	*Piper nigrum*	Piperaceae	0.276	Fruit	Fever	Pounded	Internal	0.500
						Stomach ache	Infusion	Internal	0.167
7.	Bawang putih	*Allium sativum*	Alliaceae	0.207	Bulb	Itchy	Pounded	Internal	0.143

						Fever	Pounded	Internal	0.500
						Convulsions	Pounded	External	0.667
8.	Jagung	*Zea mays*	Poaceae	0.138	Fruit	Skin disease	Pounded	External	1.000
9.	Alang-alang	*Imperata cylindrica*	Poaceae	0.138	Shoots	Eye pain	Direct application	External	0.556
						Rheumatic pain	Infusion	Internal	0.545
10.	Adas	*Foeniculum vulgare*	Apiaceae	0.138	Seed , Leaves	Itchy	Pounded	External	0.143
						Bloating	Pounded	External	0.500
						Wound	Infused	Internal	0.500
11.	Terong tengger	-	Solanaceae	0.103	Fruit	Rheumatic pain	Infusion	Internal	0.545
						Thrush	Direct ingestion	Internal	1.000
12.	Temu ireng	*Curcuma aeruginosa*	Zingiberaceae	0.103	Rhizome	Itchy	Pounded	Internal	0.143
13.	Simbukan	*Paederia foetida*	Rubiaceae	0.103	Leaves	Bloating	Direct ingestion	External	0.500
						Fever	Decoction	Internal	0.500
14.	Semanggi	*Marsilea crenata*	Marsileaceae	0.103	Leaves	Cough	Infusion	Internal	1.000
15.	Ketirem			0.103	Leaves	Hypertension	Infusion	Internal	0.167
16.	Ciplukan	*Physalis angulata*	Solanaceae	0.103	Fruit	Hypertension	Infusion	Internal	0.167
						Wound	Pounded	External	0.500
						Stomach ache	Infusion	Internal	0.167
17.	Binahong	*Anredera cordifolia*	Basellaceae	0.103	Leaves	Wound	Pounded	External	0.500
						Stomach ache	Infusion	Internal	0.167
18.	Alpukat	*Perseaamericana*	Lauraceae	0.103	Leaves, Fruit	Hypertension	Infusion	Internal	0.167
19.	Pronojiwo	*Sterculiajavanica*	Malvaceae	0.069	Seed	Stomach ache	Pounded	Internal	0.167
20.	Mawar	*Rosa sp.*	Rosaceae	0.069	Flower	Itchy	Direct application	external	0.143
21.	Kelapa hijau	*Cocos nucifera*	Arecaceae	0.069	Fruit	Fever	Pounded	Internal	0.500
22.	Bayam	*Amaranthus roseus*	Amaranthace-ae	0.068	Leaves	Fever	Squeezed	External	0.500
							Decoction		
23.	Mahoni	*Swietenia mahagoni*	Meliaceae	0.034	Fruit	Hypertension	Direct ingestion	Internal	0.167
24.	Seledri	*Apium graveolens*	Apiaceae	0.034	Leaves	Hypertension	Infusion	Internal	0.167
25.	Beluntas	*Pluchea indica*	Astraceae	0.034	Leaves	Itchy	Pounded	External	0.143
26.	Sempretan	*Bidens pilosa*	Asteraceae	0.034	Leaves	Itchy	Infusion	Internal	0.143
27.	Tales bentol	*Colocasia esculenta*	Araceae	0.034	Bark	Wound	Direct application	External	0.500

28.	Tiris	*Kalanchoe pinnata*	Crassulaceae	0.034	Leaves	Wound	Squeezed	External	0.500
29.	Pisang pendek	*Musa paradisiaca*	Musaceae	0.034	Latex	Wound	Direct application	External	0.500
30.	Labu siam	*Sechiumedule*	Cucurbitaceae	0.034	Fruit	Stomach ache	Maceration	Internal	0.167
31.	Pala	*Myristica fragrans*	Myristicaceae	0.034	Fruit	Fever	Pounded	Internal	0.500
32.	Dadap serep	*Erythrina variegata*	Fabaceae	0.034	Leaves	Fever	Decoction Maceration	Internal	0.500
33.	Krokot	*Portulaca*	Portulaceae	0.034	Leaves	Fever	Maceration	Internal	0.500

Table 2. List of medicinal plants used as traditional Tengger Tribe health care medicine (cont'd)

No	Local name	Scientific name	Family	UV	Part use	Medicinal use	Preparation	Administration way	ICF
34.	Grinting	-	-	0.034	Leaves	Fever	Maceration	Internal	0.500
35.	Sirih	*Piper betle*	Piperaceae	0.034	Leaves	Eye pain	Maceration	External	0.556
36.	Lobak tengger	*Raphanus raphanistrum*	Brassicaceae	0.034	Bulb	Stomach ache	Infusion	Internal	0.167
37.	Kayu putih	*Melaleuca leucadendra*	Myrtaceae	0.034	Leaves	Stomach ache	Maceration	External	0.167
38.	Jambu jingo	*Psidium guajava*	Myrtaceae	0.031	Fruit	Diarrhea	Direct ingestion	Internal	0.800

Kunir or turmeric (*Curcuma longa*) is one of the plants that becomes common traditional medicinal ingredient of the Tengger tribe for the treatment of cough, itchy, wound, fever, dizziness and stomach ache. The data is in accordance with the results of the previous study. *C. longa* had various pharmacological effects, such as antifungi (Misra and Sahu, 1977), antibacterial (Shankar and Murthy, 1979), anti-inflammatory (Ghatak and Basu, 1972), anticoagulant (Srivastava et al., 1985) and gastroprotectant (Lee et al., 2003). *C. longa* could reduce symptoms of respiratory diseases, such as dyspnea, cough and sputum (Jain and DeFillips, 1991). The curcumin and fraction of oil from *C. longa* could suppress the growth of some bacteria such as *Streptococcus*, *Staphylococcus* and *Lactobacillus.* The ethanolic extract, ether extracts, chloroform extracts, and turmeric oil had antifungal activity against *Aspergillus flavus*, *A. parasiticus*, *Fusarium moniliforme* and *Penicillium digitatum*. Antiinflammatory activity of curcumin in *C. longa* was shown by its effectiveness against the induction of carrageenan edema in mice because curcumin could increase the mucin secretion in rabbits and might act as a gastroprotectant against irritation (Lee et al., 2003).

Jahe or ginger (*Zingiber officinale*) ranked in the second place as a plant that was considered important by the Tengger Tribe community. Empirically, *Z. officinale* were used in the treatment of coughs and stomach aches in the Tengger tribe community. *Z. officinale* rhizomes can be used for the treatment of fever, cough and stomach ache based on the chemical compounds contained, such as guanicol as a cough medicine, limonene as a cold medicine and

1.8-cineole as a stimulus for sweating, fever, dizziness and cold. Based on the research carried out by Zakaria and Rajab (1999), *Z. officinale* rhizome extract could enhance the immune system because stimulated lymphocyte proliferation, increased the phagosity activity of macrophages and the activity of natural killer cells that can lyse tumor cells and virus infected cells.

Tepung otot (*Stellaria media*) is a plant that commonly known as invasive weed in gardens and fields. However, this plant was used by the Tengger Tribe community as a medicinal ingredient in medication for sprains, rheumatic pain and wounds. *S. media* contains steroids, flavonoids, tannins, carbohydrates, proteins and fats (Arora and Sharma, 2012). Based on Oyebanji et al. (2012), methanol extract of *S. media* had anti-inflammatory and analgesic activity. Methanolic extract from *S. media* could mediate antinflammatory and analgesic in the peripheral and central nervous system by inhibiting histamine, serotonin, kinin, prostaglandin and cyclooxygenase release.

CONCLUSION

Based on the data analysis of ethnopharmacy study to the Tengger tribe in Ledokombo and Pandansari villages Sumber district Probolinggo regency, it was known that there were 38 species of plants used by the Tengger people as traditional medicines. The method of processing plants as of traditional medicine ingredients was by raw crushing in water, boiling, consuming them directly, soaking, brewing, squeezing and burning. Meanwhile, the way to use the traditional medicines were by drinking, smearing, pasting and eating. The diseases that often suffered by Tengger people based on ICF values were thrush, skin disease, dizziness, sprain, cough and diarrhea. The plants that were considered important by the Tenger tribe based on the UV were *Curcuma longa*, *Zingiber officinale* and *Stellaria media*.

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The Evaluation of Rationality of Antibiotic Administration to Inpatients with Urinary Tract Infection at Salatiga Regional General Hospital in June 2017 - December 2017

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Abstract

Infection is one of serious public health problems particularly in developing countries. One of the most common infections is urinary tract infection (UTI). UTI is a condition where there are large numbers of microorganisms in the urine that cause infections. Common treatment therapies used to treat infectious diseases are antibiotics with the purpose of preventing more severe infections, eradicating infectious microorganisms and preventing the recurrence. Improper use of antibiotics can cause ineffective treatment, decreasing of drug safety, increasing of resistance, and increasing of medical costs. This study aims to determine the quality of antibiotic use using the Gyssens flowchart applied to patients suffering from urinary tract infections at the Salatiga Regional General Hospital for in June 2017 - December 2017. This research is an observational study where the data collection was done retrospectively. The data were analyzed with Gyssens flowchart and then compared with European Association of Urology Guidelines on Urological Infections in 2015. The results showed that there were antibiotic prescribing way which were included in category IVC (38.62%), category IIIA (6.21%), category IIIB (6.89%), category IIA (0.69%), category I (2.07%), category 0 (45.52%), and none of the these prescribing ways were included in the following categories: IIB, IIC, IVA, IVB, IVD, V and VI. Antibiotics that were often given are ceftriaxone (39.31%), cefixime (16.55%) and ciprofloxacin (11.03%).

Keywords : Antibiotics, Urinary Tract Infection

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INTRODUCTION

Urinary tract infection (UTI) is a condition where there are a large number of microorganisms in the urine, and they are capable of causing infection in the urinary tract. To state that UTI exists, bacteria must be found in urine (Izrar, 2009).

The main therapy for UTI is antibiotic therapy with the aim of preventing more severe infections, eradicating infectious microorganisms, and preventing the recurrence. Management of antibiotic therapy for UTI is based on the types of bacteria, the signs and symptoms experienced by the patients, the location of the infection (lower or upper urinary tract infection), and the clinical condition of the infection (complex or simple).

Antibiotics are chemical substances produced by fungi and bacteria that are able to kill germs or inhibit their growth while, on the other hand, the toxicity of humans is relatively small (Indijah and Fajri, 2017).

According to the WHO research (2014), urinary tract infection is one of the infections having a high proportion as the contributor to antibiotic resistance in the world; hence considering rational use of antibiotics. Drug uses are rational if they fulfill the criteria of right indications, right patient, right drug selection, right dose, right time interval of administration, right duration of administration, and right assessment of the patient's condition (Ministry of Health, 2011).

In hospitals, unnecessary or excessive use of antibiotics encourages the development of resistance and multiplication of resistance to certain bacteria that will spread through cross infection. Antibiotic resistance control can reduce the incidence of antibiotic resistance, prevent toxicity due to the use of antibiotics, reduce the cost of improper use of antibiotics, and reduce the risk of nosocomial infections (Ministry of Health, 2011).

The Salatiga Regional General Hospital (RSUD) is the regional hospital in the city of Salatiga which has many urinary tract infection patients (Anonim, 2017). The large number of inpatients with UTI triggers the frequent use of antibiotics in order to deal with infections.

One way to find out the rationality of antibiotic use is the Gyssens method (Ministry of Health, 2015). The Gyssens method is a flowchart that has been widely used to evaluate the quality of antibiotic use. This method assesses the accuracy of the antibiotics use such as the accuracy of indication, accuracy of selection based on effectiveness, toxicity, price, spectrums, duration of administration, doses, intervals, routes, and time of administration.

From the aforementioned description, the researcher considers that it is necessary to conduct a research on the rationality of antibiotic use for hospitalized patients with urinary tract infections in the Salatiga Regional General Hospital.

MATERIALS AND METHODS

Research Design

This research is a non-experimental, descriptive study that explains how the suitability of antibiotic administration, for hospitalized patients suffering from UTI, was compared to the European Association of Urology Guidelines on Urological In 2015. The data were collected retrospectively, namely by observing and evaluating UTI patients' medical records taken from the population of medical records of inpatients at Salatiga Regional Hospital during the period of June 2017 to December 2017.

Population, Samples and Sampling Techniques

The population in this research was medical records of hospitalized patients in Salatiga Regional Regional Hospital. The research samples were taken from the medical records of hospitalized patients with UTI in June 2017 - December 2017. The sampling technique in this research was the purposive sampling system. This sampling system took all medical records of UTI inpatients in Salatiga Regional General Hospital in June 2017 until December 2017.

Criteria for inclusion and exclusion

The sample inclusion criteria were patients treated in the inpatient wards of Salatiga Regional General Hospital during June 2017 - December 2017, the patients were the ones suffering from urinary tract infections and patients who received antibiotic therapy. Exclusion criteria were patients who were admitted to ICU and HCU, patients who passed away and incomplete medical record data.

Variable Operational Definitions

1. Antibiotic prescribing is written antibiotic requests from doctors to the pharmacy installation of Salatiga Regional General Hospital which are then given to inpatients in Semarang Regional General Hospital.
2. Patients with urinary tract infections who received antibiotic therapy and were treated in the inpatient wards of Salatiga Regional General Hospital.
3. There is a possibility that patients are treated with more than one diagnoses and get antibiotic therapy as well.

Research Instruments

The instruments used in this research were Guidlines on the 2015 Europen Association of Urology Urological Infections (Grabe et al., 2015), Gyssens flowcharts, and the samples used were Medical Records of hospitalized patients with urinary tract infections in the Salatiga City Hospital during June 2017 - December 2017.

The Research Steps

1. Data collection
2. Data selection
3. Presentation of data
4. Evaluation of data
5. Conclusions

Data analysis

Researchers took medical record samples of inpatients with urinary tract infections in Salatiga Regional General Hospital in the period of June 2017 - December 2017. Data collected from these samples were then listed into the patient's antibiotic recap tables. Next, the quality of the collected data were assessed using Gyssens flowcharts. The assessment results;thus, described the number of samples in each category divided by the total number of samples and multiplied by 100% to obtain the percentage of each category. From the obtained data, it can be concluded that the rationality level of antibiotic administration to inpatients with urinary tract infections the hospital from June 2017 until December 2017.

RESULT AND DISCUSSION

Result

Based on the Inpatient Index Card of Salatiga Regional General Hospital in June 2017 - December 2017, there were 119 medical records of patients diagnosed with Urinary Tract Infection. Of the 119 samples, there were 25 samples included in the exclusion criteria; namely, one sample was incomplete and 24 samples that did not get antibiotic therapy; hence only 94 samples were used. Each sample of medical records which were examined was given a code number so there were samples with code number 1 to 94.

From the 94 medical record records fulfilling the inclusion criteria, there were 145 antibiotics which were used since one patient can get more than one antibiotic therapy. The patients underwent parenteral therapy and then oral therapy.

Discussion

1. Characteristics of the Patients Based on Gender

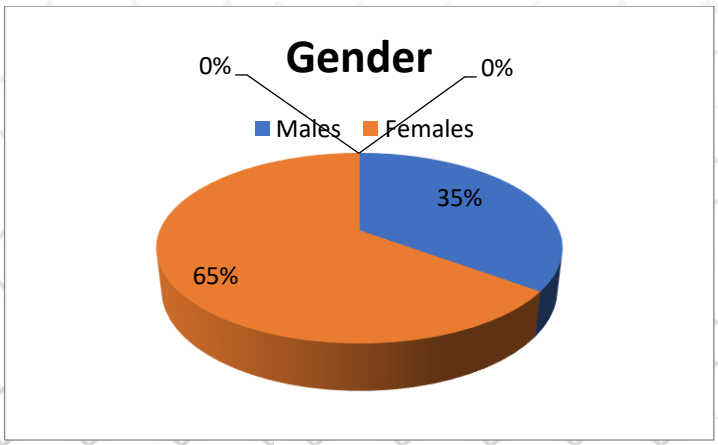

Figure 1. Characteristics of UTI Patients in Salatiga Regional General Hospital Based on Gender during June 2017-December 2017.

From the diagram, it can be seen that the incidence of UTI in women is more frequent than that of men in which there were 61 female patients (65%) and 33 male patients (35%). The reason of this condition is that women have shorter urethra (2-3 cm) and the urethras are located close to the perianal and vaginal areas so that microorganisms from the outside are easier to reach the bladder, especially E. coli bacilli. Men, however, beside they have longer urethra r (15-18 cm), their prostate fluid also has bactericidal properties which are protective against infections caused by uropathogenic bacteria (Purnomo, 2011)

2. Characteristics of Patients According to their Age

This study classified patients into five age groups, namely the infant group for patients at the age of 1 month - 2 years, children for patients at the age of 3-11 years, adolescents for patients at the age of 12-18 years, adults for patients at the age 19-65 years, and geriatricsfor patients at the age of more than 65 years (Barker C and Nunn AJ, 2003)

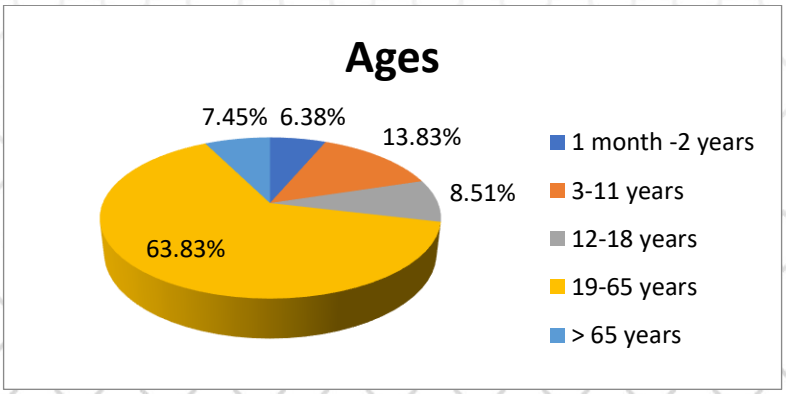

Figure 2. Characteristics of UTI Inpatients Based on their Ages in Salatiga Regional General Hospital from June 2017 - December 2017.

The diagram shows that based on age, the highest percentage of UTI patients occurred at the age of 19-65 years (adults) as much as 63.83%. Perhaps this phenomenon is caused by an infection and a decrease in urinary tract function. Unhealthy sexual activity often causes bacteria to enter the urinary tract (Nofriaty, 2010).

Urinary tract infections that occured in infants at Salatiga Regional General Hospital were 6.38%. Although it is in the lowest position, it indicates that the baby can experience a urinary tract infection. The Indonesian Paediatricians Association in the Consensus of Urinary Tract Infection in Children states that UTI needs to get the attention from doctors and parents due to various reasons, one of them is that frequent UTI can often be a sign of abnormalities of kidneys and urinary tracts such as Reflux Vesiko Ureter (RVU) or obsrtuctive uropathy (Dr. Sudung O. Pardede, SpA et al., 2011). Usually babies with UTI do not show specific symptoms, so babies with high fever for more than 3 days are usually examined at laboratories diagnoses including the diagnosis of UTI.

Urinary tract infections often also occur in geriatric patients (> 65th). These cases happened because within the elderly period, the number of bacteria will increase significantly from 5% - 10% to 20% at the age of 70 years, and it will continue to increase in line with their ages (Purnomo, 2011).

3. The Description of Antibiotics Prescribing

In this research, from the 94 medical records, there were 145 antibiotics used. The description of antibiotic use for inpatients with urinary tract infections at Salatiga Regional General Hospital from June 2017 to December 207 can be seen from the following diagram:

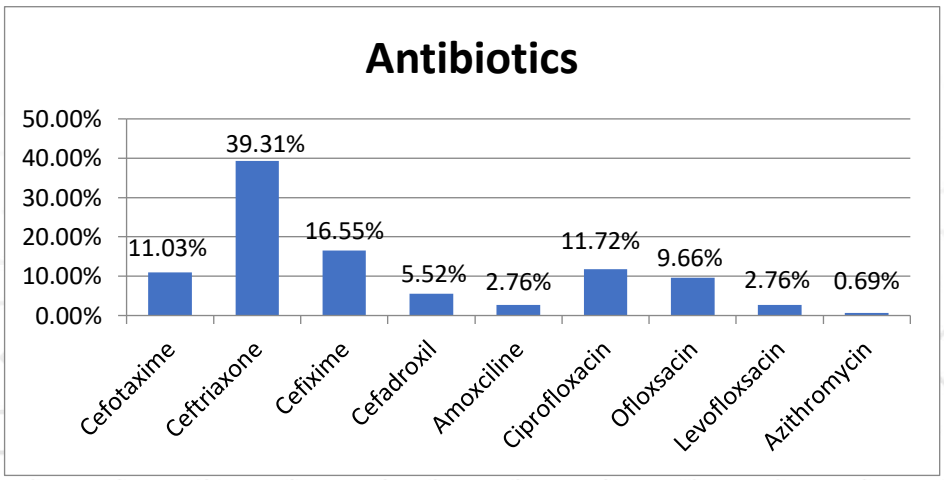

Figure 3. The Use of Antibiotics for patients of UTI hospitalized in Salatiga Regional General Hospital fro June 2017 to December 2017.

Figure 3 shows that the antibiotics mostly used for the treatment of inpatients with urinary tract infections in Salatiga Regional General Hospital during the period of June 2017 until December 2017 were ceftriaxone (39.31%), cefixime (16.55%), and ciprofloxacin (11.03%).

In accordance with Guidelines on Urological Infections, ceftriaxone and cefixime, the third generation cephalosporin antibiotics, are the choice for empirical therapy of urinary tract infections (Grabe et al., 2015).

Ciprofloxacin is included as fluoroquinolone class commonly used for infections caused by E. coli (Ministry of Health RI, 2011). E. coli bacteria are the most common microorganism that causes urinary tract infections (Yulianto, 2009); therefore, ciprofloxacin is widely used as the treatment option for urinary tract infections in Salatiga Regional General Hospital.

4. Antibiotic Evaluation Based on Gyssens Flowchart

In this research, the quality of antibiotic administration for hospitalized patients with urinary tract infections was assessed using Gyssens flowchart. The assessment of antibiotic quality with Gyssens flowchart was divided into 0-VI categories. Based on the results of the analysis of 145 antibiotics given to 94 inpatients with Urinary Tract Infection in Salatiga Regional General Hospital during the period of June 2017 to December 2017, there were ways of prescribing that fell into several categories:

a. Category 0 : The use of antibiotics is proper/careful.
b. Category I : The use of antibiotics is not in the right timely.
c. Category IIA : The use of antibiotics is not in the right dose.
d. Category IIB : The use of antibiotics is not in the right interval of administration.
e. Category IIC : The use of antibiotics is not in the right method/route of administration.
f. Category IIIA : The use of antibiotics is too long.
g. Category IIIB : The use of antibiotics is too short.
h. Category IVA : There are other more effective antibiotics.
i. Category IVB : There are other less toxic/safer antibiotics.
j. Category IVC : There are other more affordable antibiotics.
k. Category IVD : There are other antibiotics with narrower spectrums.
l. Category V : There is no indication of antibiotic use.
m. Category VI : Medical record data is incomplete and cannot be evaluated.

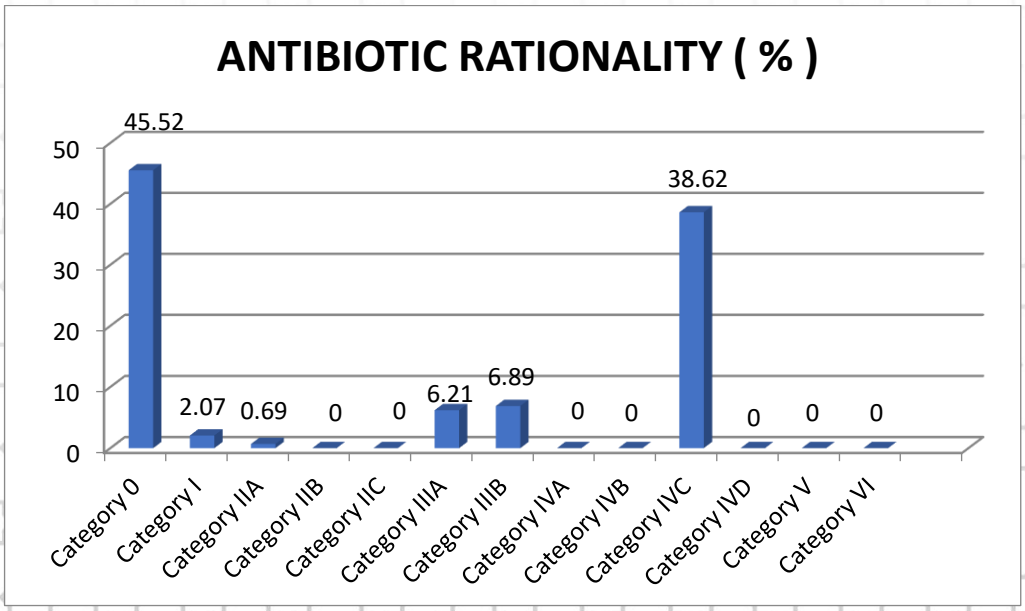

Figure 4. The Diagram of the Quality Assessment Percentage of Antibiotics Use for UTI inatients at Salatiga Regional General Hospital from June 2017 - December 2017 Based on the Gyssens Flowchart.

Figure 4 shows the percentage of quality assessment of antibiotic use for inpatients at Salatiga District Hospital from June 207 - December 2017 based on Gyssens flowchart in which there were 66 prescriptions (45.52%) categorized as 0 which means appropriate for the patients; needs (based on safety, suitability, and the costs needed for therapies, dosism interval duration, route and time of administration). There were 3 (2.07%) prescriptions from 94 patients included in category I where antibiotics were not given at the right time (Table 1). The administration of antibiotics is correct providing given at the same time from the first day.

There was 1 (0.69%) prescription included in the IIA category because the given antibiotics were not in the right dose. The right dose takes account of the patient's weight and the patient's age. In the IIIA category, there were 9 prescriptions (6.21%) in which ofloxacin and ciprofloxacin were administered too long (Table 2). According to the 2015 European Association of Urology Guidelines on Urological Infections, the duration of administration of ofloxacin and ciprofloxacin for UTI patients is for 3 days. In the IIIB category, 10 (6.89%) prescribing methods,cefatoxime and ciprofloxacin administration, were too short (Table 3). The antibiotic administration may stop due to allergies, forcible dischcharge, or improving condition but not continuing to oral therapy.

There were 56 (38.62%) prescriptions included in the IVC category, namely the appropriate but unwise use of antibiotics because there were more affordable choices of antibiotics. The kind of antibiotics included in this category are cefriaxone injection, Lapoxime injection and Lapicef syrup.

From the 145 antibiotics prescribed to the 94 patients, none of them stopped in the following cateegories: IIB, IIC, IVA, IVB, IVD, V, and VI.

Table 1. Antibiotics for Inpatients with Urinary Tract Infection in Salatiga Regional General Hospital from June 2017 to December 2017 included in Category I of Gyssens Method

Code	Name	Regimens	Administration time
72	Azithromycin	1 x 500 mg (every 24 hours)	21.00, 10.00, 21.00
56	Ceftotaxime	2 x 1 gram (every 24 hours)	17.00, 21.00 09.00, 21.00 09.00, 21.00
81	Ceftotaxime	1 x 1 gram (every 24 hours)	15.00 09.00 09.00

Table 2. The use of antibiotics in category IIIA based on Gyssens flowchart for inpatients with UTI at Salatiga Regional General Hospital from June 2017-December 2017.

Patients' Codes	Names of Antibiotics	Duration (Days)
2,80	Ofloxsacin 200mg	8
3	Ofloxsacin 200mg	12
19,32,87	Ofloxsacin 200mg	5
73	Ofloxsacin 200mg	4
46	Ofloxsacin 200mg	7
74	Ciprofloxacin 500mg	4

Table 3. The use of antibiotics in category IIIB based on Gyssens flowchart for inpatients with UTI at Salatiga Regional General Hospital from June 2017-December 2017.

Patients' Codes	Names of Antibiotics	Duration (Days)
9	Ceftotaxime	2
10	Ceftotaxime	4
14	Ceftotaxime	1.5
16	Ceftotaxime	3
17	Ciprofloxacin	1

36	Ciprofloxacin	2
60	Ceftotaxime	5.5
61	Ceftotaxime	2
79	Ceftotaxime	3
12	Ceftotaxime	1.5

CONCLUSION

Based on the discussion related to the data analysis, it can be concluded that the use of antibiotics for inpatients suffering from Urinary Tract Infection in Salatiga Regional General Hospital from June 2017 to December 2017 is not totally rational. The examination of antibiotic use quality employing the Gyssens method showed that from 94 patients, there were antibiotic prescribing included in the category IVC (38.62%), category IIIA (6. 21%), category IIIB (6.89%), category IIA (0.69%), category I (2.07%), category 0 (45.52%), and none of them were included in category IIB, category IIC, category IVA, category IVB, category IVD, category V, and category VI.

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Evaluation of Use of Hepatitis B Drugs in Hospitalized Patients at "X" Hospital Semarang in the Period from January 2015 to December 2016

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Abstract

Background: Hepatitis B is a liver disease caused by the hepatitis B virus which can cause acute or chronic liver inflammation that can lead to liver cirrhosis or liver cancer. Patients with hepatitis B virus infection can be given hepatoprotector drugs, cholelitolytic drugs and antiviral drugs. The purpose of this study was to determine the characteristics of hepatitis B patients and evaluation of the use of each hepatitis B drug in hepatitis B patients. The evaluation includes appropriate indication, appropriate medicine, appropriate patient, appropriate dosage, and evaluation of potential drug interactions during therapy.

Methods: This study was an observational study, with a cross-sectional design. Data retrieval is done by purposive sampling. Inclusion criteria are patients with acute hepatitis or chronic age 26-45 years who have been approved laboratory tests, with or without complications. The type of qualitative data includes the type of drug and the dose given, then compared with the literature to assess accuracy. Results of the assessment of conformity with the literature and accuracy verified in percent.

Results: The results obtained 61 patients who met the inclusion criteria with characteristics based on sex were found to be the most common in males (73.77%) and age range 41-45 years (37.70%) at the most. Evaluation of the use of each hepatitis B drug in this study obtained results: appropriate indication for hepatoprotector drugs (100%) and antiviral drugs (81.97%), appropriate medicine (100%), appropriate patient (100%), and appropriate dosage (100%). There is 1 type of potential drug interaction in the treatment of hepatitis B with other drugs, namely between alpha 2-a interferon and ondansetron as many as 3 cases (4.92%).

Keywords: Hepatitis, Hepatitis Drugs, Accuracy, Potential Interactions

INTRODUCTION

Hepatitis B is a liver disease caused by hepatitis B virus. (1) Transmission of hepatitis B occurs through contact with the patient's body fluids. If the disease is not handled properly, it can cause chronic liver disease and even cirrhosis of the liver and liver cancer which is at high risk of causing death. (2)

Patients with acute hepatitis B only need supportive care. (3). Antiviral therapy is indicated for hepatitis B fulminant, severe hepatitis, jaundice with bilirubin> 10 mg / dL, infection along with the presence of hepatitis C or D. virus (4). The use of interferon can increase the risk of hepatic necro-inflammation, so it needs to be avoided. An acceptable choice for chronic hepatitis B is tenofir, telbivudine, and entecavir nucleoside analogues given monotherapy in short-term use (4,5) and lamivudine, adefovir, dipivoxil. (5) In April 2018, CNN Indonesia reported that a study published in the journal Lancet Gastroenterology and Hepatology, only 1 in 20 people contracted HBV who received appropriate medical treatment. Data on the price of antiviral drugs for hepatitis B can reach 500 thousand - 1 million per month.

Based on a number of things above, it is necessary to do this research to determine the accuracy of drug use in terms of indications, drugs, patients, dosages and potential drug interactions and patterns of drug use in hepatitis B.

METHODS

This study was an observational study with a cross-sectional design. The data was taken retrospectively at the "X" hospital in Semarang, the material used was medical records. The data period used is January 2015-December 2016. The types of qualitative data include the type of drug and the dose given.

The sample size is based on the inclusion criteria. Subjects were patients with acute and chronic hepatitis B, with complications and non-complications. The inclusion criteria are patients aged 26-45 years old, undergoing laboratory examinations and receiving drugs, and complete medical record data. Exclusion criteria were patients with HBsAg non reactive laboratory examination results.

The variables in this study are the accuracy of the indication, drug, patient and dosage and the potential for drug interactions. To assess accuracy is done by recording the condition and drug data received by the patient, then matched with the literature. Evaluation of the potential for drug interactions is done by looking at the data of drugs that are used simultaneously, then matched with the literature. Explanation to evaluation results is carried out descriptively, referring to the libraries used. The results of the conformity assessment with the literature and

accuracy are expressed in percent. This research was conducted without meeting patients, so there were no ethical tests or requests for informed consent.

RESULTS

A total of 61 patient data met the inclusion criteria, the description data of the use of hepatitis B drugs used in the hospital "X" can be seen in Table 1. The result in Table 1 shows that all patients received hepatoprotector curcuma, while antivirals were given telbivudin (11.75% 0 and peginterferon α2 (9.8%).

In Table 2 shows the use of drugs based on patient diagnosis. There is a difference in drug administration given to hepatitis without complications with hepatitis + complications. The ones without complications are given at most 2 types of drugs, while with complications at least 2 types of drugs are given.

The data obtained are then evaluated for the accuracy of the indications, drugs, patients, and doses where the results of the evaluation of accuracy can be seen in Table 3. Four kinds of drugs are given in cases of hepatitis, curcuma drugs, ursodeoxycholic acid, and peginterferon α2 exactly 100% for indication, medication, patient, and dosage. A drug, telbivudin, medicine, patient, and a dose of 100%, while the exact indication is 82%.

The data shown in Table 4 shows the potential occurrence of drug interactions, namely drugs taken simultaneously by the patient. There are 3 cases (4.92%) giving 2 kinds of drugs that are given simultaneously (Peginterferon alfa 2a with ondansetron) which has the potential to cause interaction.

DISCUSSION

Overview of drug use

All patients (100%) received the curcuma hepatoprotector drug, while the Wenge (2009) study showed 81% of patients received hepatoprotector (curcuma 9.5%) and in the Trisnaningtyas (2017) study patients who received hepatoprotector were 33.52% (curcuma 17 , 88%). The difference in the percentage of curcuma administration in this study is different from the previous study because the research sites were different hospitals and cities.

Hepatoprotector administration in hepatitis B patients is intended to restore liver function to improve again so that the liver parameter values, one of which is ALT / AST, returns to normal. (6) The administration of curcuma as a hepatoprotector is intended to prevent liver cell damage. (7) Based on research, curcuma can inhibit hepatitis B virus infection (8)
Distribution of Ursodeoxycholic acid in hepatitis B patients, due to Ursodeoxycholic acid significantly reduces the risk of hepatitis B antigen positivity (HBsAg) and serum DNA level

of hepatitis B virus. Ursodeoxycholic acid significantly reduces the risk of an increase in abnormal serum transaminase activity in chronic hepatitis B. (9) The provision of pegylated interferon alpha antiviral namely alpha 2-a interferon in hepatitis B patients aims to increase the host immune response to fight hepatitis B virus and inhibit hepatitis B virus replication. (10) In the studies conducted by Wenge (2009) and Trisnaningtyas (2017) there were no patients who were prescribed the drug.

Distribution of nucleoside analogues in hepatitis B patients due to nucleoside analogues effectively suppresses the level of DNA of hepatitis B virus. (5) Telbivudin is one of the nucleoside analog class drugs that function in inhibiting hepatitis B virus replication (11). The evaluation results of the use of hepatitis drugs were 100% of patients with hepatoprotector curcuma administration, and 81.97% of antiviral drugs. Therapy given is in accordance with the PPHI (2009) guideline, including the exact drug and dosage of 100%. Indications of curcuma administration in accordance with the Indonesian Depkes RI (2008) are exactly 100%, while indications of antiviral administration based on PPHI are exactly 82%.

Overview of drug use based on disease diagnosis

Based on the results of hepatoprotective combination treatment and antiviral drugs compared to non-hepatoprotective antiviral drugs in hepatitis B patients showed that combination therapy of hepatoprotective agents and antiviral drugs was more effective than anti-hepatoprotective antiviral drugs to reduce liver cell damage and the use of two hepatoprotective agents was better than hepatoprotective agents single in normalizing aminotransferase (ALT) levels and the amount of bilirubin in hepatitis B patients (12)

As a comparison for the evaluation of therapy for hepatitis B patients at Dr. Sardjito Hospital Yogyakarta in 2012-2014, the antiviral drugs used were entecavir by 4%, lamivudin by 88%, and tenofovir by 8%. Supporting therapies used in hepatitis B therapy as hepatoprotectors were curcuma at 17.88%, SNMC at 8.38%, and HP Pro at 7.26%. (6) Based on studies of the characteristics and use of drugs in patients with hepatitis B in Manado City Government Hospital for the period January 2011-December 2012, the drug for hepatitis B treatment used was hepamax by 38.6%, curcuma by 30%, pro liver by 1.4 %, sebivo by 5.7%, lamivudin by 1.4%, HP Pro by 5.7%, lesichol 300 by 1.4%, HP Pro + hepamax by 5.7%, curcuma + hepamax by 12.9% , and hepamax + sebivo at 1.4%. (13) So, there are differences in the delivery of hepatitis B drugs to hepatitis B patients in each of these hospitals.

Appropriate evaluation of indications

Patients with hepatitis B virus infection can be given hepatic protector drugs and cholelitolytic drugs. (14) Curcuma can be indicated in patients with hepatitis B because it has an antioxidant effect by capturing superoxide ions and breaking the chain between superoxide ions so as to prevent liver cell damage. (7) Curcuma can inhibit hepatitis B virus infection by inhibiting histone acetylation of hepatitis B virus-cccDNA bonds which results in the positivity of hepatitis B surface antigen (HBsAg) and reduced activity of hepatitis B virus (HBeAg) so that

hepatitis B virus replication is inhibited. (8) Ursodeoxycholic acid can be indicated in hepatitis B patients because it can reduce ALT levels in hepatitis B patients (12) by increasing the transport of bile acids and / or detoxification, cytoprotection and anti-apoptotic effects. (15)

In the literature referred to from (16, 17, 14) does not explain the handling of hepatitis B patients with hepatocellular carcinoma (hepatoma). However, in the research journal it was explained that hepatitis B patients accompanied by hepatocellular carcinoma should be considered for the provision of antiviral therapy in order to prevent tumor recurrence or prevent liver decompensation. (18)

Appropriate evaluation of drugs

(14) Curcuma has the effect of being an antioxidant capable of capturing superoxide ions and breaking the chain between superoxide ions, thus preventing liver cell damage. (7) Based on curcuma research, it can inhibit hepatitis B virus infection by inhibiting histone acetylation of hepatitis B virus-cccDNA bonds which results in the positivity of hepatitis B surface antigen (HBsAg) and decreased activity of hepatitis B virus (HBeAg) so that hepatitis B virus replication is inhibited. (8) The administration of hepatoprotectors in hepatitis B patients is intended to restore the liver's function so that it returns to recovery so that the liver parameter values, one of which ALT / AST can also return to normal. (6)

Ursodeoxycholic acid is clinically included in hepatoprotector agents, can be indicated in hepatitis B patients because it can reduce ALT levels in hepatitis B patients (12) by increasing the transport of bile acids and / or detoxification, cytoprotection and anti-apoptotic effects. (15) Based on Ursodeoxycholic acid studies significantly reduce the risk of hepatitis B surface antigen positivity (HBsAg) and hepatitis B virus serum DNA levels for acute hepatitis B and ursodeoxycholic acid significantly reduce the risk of increased abnormal serum transaminase activity in chronic hepatitis B. (9)

Nucleoside analogues can be given to hepatitis B patients, nucleoside analogues effectively suppress hepatitis B virus DNA levels. (5) Telbivudin functions in inhibiting hepatitis B virus replication. from ALT normalization. (19) In a comparative study of telbivudin and entecavir, both drugs provided the same potential in normalizing ALT and suppressing the hepatitis B virus DNA in short-term therapy. In the case of HBeAg telbivudin clearance is superior to entecavir (20) In another study it was also found that the administration of telbivudin was superior to adefovir in suppressing the hepatitis B virus DNA until it was not detected. (16)

As many as 100% of patients receiving alpha 2-a interferon injections are right for the drug. Pegylated interferon alpha has a dual mechanism of action as an immunomodulator in which pegylated interferon alpha activates macrophages, natural killer cells (NK) and cytotoxic T lymphocytes and modulates the formation of antibodies that will enhance the host's immune response to fight hepatitis B virus and as antiviral activity by inhibiting the replication of hepatitis B virus directly through activation of endo-ribonuclease, elevation of protein kinase

and induction of 2 ', 5'-oligodenylate synthetase. (10) Pegylated interferon is available in 2 types, namely pegylated-interferon α-2a (Peg-IFN α-2a) and pegylated-interferon α-2b (Peg-IFN α-2b), based on research there are no significant differences in the use of Peg-IFN α -2a and Peg-IFN α-2b in the inhibition of hepatitis B virus replication. (21) At this time what has been accepted as a drug for hepatitis B is pegylated interferon α-2a. (10)

Appropriate patient evaluation

Curcuma is contraindicated in patients with hypersensitivity to curcuma. (26) Ursodeoxycholic acid is contraindicated in patients with hypersensitivity to Ursodeoxycholic acid. (26) In all patients there is information that there is no history of allergy to the drug.

Telbivudin is contraindicated in patients with hypersensitivity with telbivudin. (22) Interferon is contra-indicated in patients with psychiatric disorders, women who are pregnant, active autoimmune diseases. (16) Interferon alpha 2-a is also contraindicated in patients with hypersensitivity to active substances (alpha-interferon) and excipients. (23) In this study all patients had no history of allergy to the drug.

Appropriate dosage evaluation

Maximum safety indication of curcumin dose is 12g / day with 3 months treatment duration. (24) The administration of Curcuma 20 mg 3 times daily in all patients did not exceed the maximum safety indication limit of dose.

Ursodeoxycholic acid dosage range for hepatitis B virus therapy ranges from 150–900mg / day with treatment duration ranging from 3 weeks to 2 years. (25) Administration of Ursodeoxycholic acid of 250 mg 3 times daily in patients not exceeding the maximum dose limit.

Telbivudin dose is recommended at 600 mg / day. (16) duration of telbivudin administration for ≥52 weeks. (26) The administration of telbivudin for 600 mg 1x a day in patients was in accordance with the recommended dosage.

A total of 6 patients received alpha 2-a interferon for 180 µg by injection, 100% had the right dose, that is precisely the amount of drug given based on the dose received by the patient stated in the medical record compared to the literature. The dose of alpha 2-a interferonone is recommended at 180µg / week with the duration of administration of alpha 2-a interferon for 48 weeks. (10) 180 mg of alpha 2-a interferon in the patient is in accordance with the recommended dose.

The potential interactions occur are minor (harmless) between alpha 2-a and ondansetron interferoners where alpha 2-a interferon will increase the ondansetron effect by affecting the

hepatic enzyme metabolism CYP1A2. (27) Potential minor drug interactions have usually mild effects and may not require changes in therapy so that they can still be tolerated. (28)

Conclusion

The evaluation results of the use of hepatitis drugs were 100% of patients with hepatoprotector curcuma administration, and 81.97% of antiviral drugs. Therapy given is in accordance with the 2009 PPHI guideline, including the exact drug and dosage of 100%. Indications of curcuma administration in accordance with the Indonesian Ministry of Health (2008) are exactly 100%, while indications of antiviral administration based on PPHI are exactly 82%.

Hepatoprotector drug (Curcuma) is used by all hepatitis B patients, either as a single drug or in combination with other drugs, aimed at uncomplicated or complicated complications of hepatitis patients.

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ATTACHMENT. TABLE OF RESEARCH RESULTS DATA

Table 1. Overview of the use of hepatitis drugs based on drug categories

No	Drug category	Drug name	Number of patients receiving	
			n	%
1.	Hepatoprotector	Curcuma	61	100
		Ursodeoxycholic acid	49	80.33
2.	Antivirus	Telbivudin	9	11.75
		Peginterferon alfa 2a	6	9.8

Table 2. Overview of drug use in hepatitis patients undergoing hospitalization at "X" Hospital for the period January 2015 - December 2016 based on diagnosis

No	Complications category	Diagnosis	Drug group	Drug name	Number of patients receiving	
					n	%
1	Without complications	Hepatitis B	Hepatoprotector	Curcuma	12	19.67
			Hepatoprotector + cholelitolytic	Curcuma + ursodeoxycholic acid	21	34.43
2	Hepatitis + complications	+ hepatoma	Hepatoprotector + cholelitolytic	Curcuma + ursodeoxycholic acid	5	8.20
		+ ascites	Hepatoprotector + cholelitolytic	Curcuma + Ursodeoxy cholic acid	5	8.20
		+ ascites + hepatoma	Hepatoprotector + cholelitolytic	Curcuma + Ursodeoxy cholic acid	3	4.92
		+ cirrhosis + ascites	Hepatoprotector + cholelitolytic+ antivirus	Curcuma + Ursodeoxy + telbivudin	5	8.20

		nucleoside analogues			
	+ jaundice	Hepatoprotector + cholelitolytic+ antivirus nucleoside analogues	Curcuma + Ursodeoxy + telbivudin	4	6.56
	+ cirrhosis	Hepatoprotector + cholelitolytic + antiviral alpha interferon	Curcuma + Ursodeoxycholic acid + injection of peginterferon alfa 2a	4	6.56
	+ Cirrhosis + Ascites	Hepatoprotector + cholelitolytic + antiviral alpha interferon	Curcuma + Ursodeoxycholic acid + injection of peginterferon alfa 2a	2	3.28

Table 3. Description of percentage (%) accuracy of Indications, drugs, patients, and doses

No	Drug category	Drug name	Indication		Drug		Patient		Dose	
			accurate	not	accurate	not	accurate	not	accurate	not
1	Hepatoprotector	Curcuma	100	0	100	0	100	0	100	0
		Ursodeoxycholic acid	100	0	100	0	100	0	100	0
2	Antivirus	Telbivudin	82	18	100	0	100	0	100	0
		Peginterferon alfa 2a	100	0	100	0	100	0	100	0

Table 4. Overview of potential drug interactions

Drug name	Amount and percentage of events	
	n	%
Peginterferon alfa 2a with ondansetron	3	4.92

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Toksisity Analysis of Pedada Leaves (*Sonneratia Caseolaris* L.) Extracts and its Isolate on Servics Cancer Cell Line (Hela)

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Abstrack

The aim of this research is to know the cytotoxic activity of n-hexane extract, ethyl acetate, ethanol, water and pedada leaf isolate (Sonneratia caseolaris.L) on cervics cancer cells using MTT [3- (4,5-dimethylthiazol-2-yl) -2.5 diphenyl tetrazolium bromide] method. The Pedada Leaf simplicia is excreted in maceration with n-hexane, ethyl acetate, ethanol, and water solvent. The cervical cell anticancer activity test against the four extracts in vitro showed that the IC50 extract values were 26.28 µg / mL for n-hexane, 9.58 µg / mL for ethyl acetate, 2.51 µg / mL for ethanol, and 760.32 µg / mL for water. Then ethanol extract was fractionated by column chromatography with eluent (n-hexan-ethyl and chloroform-methanol) obtained 12 fractions. Toxicity test from each fractionation showed that fraction of F11 with IC50 value was 95 µg / ml with DPPH method compared with other fraction. Furthermore fractionation of F11 was fractionated by column chromatography with eluen (chloroform-methanol) so that obtained fraction of F11-1 equal to 45,85 µg / mL. Furthermore, anticancer activity test from F11-1 fraction (as isolate) obtained 1.14 µg / mL while for cisplatin is 1.06 µg / mL.

Keywords: Pedada Leaf, Anticancer, Cervical Cell, Toxicity

INTRODUCTION

Based on GLOBOCAN 2012, cervics cancer ranked 7th globally in terms of rate incidence (ranked sixth in underdeveloped countries) and 8th as the cause of death (contributing 3.2% mortality, similar to mortality due leukemia). Cervics cancer occupies the highest order in developing countries, and the order of 10 in developed countries or the order of 5 globally. In Indonesia cervics cancer ranks second of 10 most cancers based on data from Anatomical Pathology in 2010 with an incidence of 12.7%. According to estimates of the Ministry of Health of Indonesia, the number of new women with cervical cancer ranges from 90-100 cases per 100,000 population and 40 thousand cases of cervical cancer every year (Kemenkes 2015).

One of the natural ingredients that has activity as cancer kemoprevensi agent is Leaf pedada (Sonneratia caseolaris.L). Based on previous research, cytotoxic activity test of leaf spade fraction (Sonneratia caseolaris L) by BSLT method where peadada leaf contains bioactive compound such as flavonoids, phenol, terpenoid and tannin. Pedada leaves have potential as anticancer. This can be seen from the test by BSLT method, the value of LC50 obtained by methanol fraction of soninalatia caseolaris leaf is 22,38 ppm. Ethyl acetate fraction 24,89 ppm and n-hexane fraction 54,83 ppm, cytotoxic activity is characterized by IC50 value of three extracts smaller than 1000 ppm. (Yulianis, 2016).

The main target of cancer therapy in general is to inhibit the growth of cancer cells through the induction of the process of cell death in apoptosis. Therefore, further research is needed to isolate the active component in the leaf extract of pedada (Sonneratia caseolaris.L) and to test its cytotoxic activity on cervical cancer cells.

Data collection technique Leaf spin extract (Sonneratia caseolaris L). The maceration tested the toxicity activity of cervical cancer cells by looking at IC50 values**.** Data analysis design Result of data obtained from toxicity test of cervical cancer cell from extract and pedada leaf isolate (Sonneratia caseolaris L). As well as calculate IC50 values obtained then analyzed by calculation and curve IC50 activity toxicity of cervical cancer cells

DISCUSSION

Cancer is a cell disease characterized by loss of cell control function to cellular regulation and homeostatic cell function in multicellular organisms. With such failure, cells can not proliferate normally. As a result, cells will proliferate continuously resulting in abnormal tissue growth (Sugiyarto, 2013).

Cancer of the cervix or cervix is a disease caused by malignant processes that occur in the cervix or cervix. The cause of cervical cancer is known is the HPV virus (Human Papilloma Virus). Cervical cancer is one of the many diseases that cause death. Efforts to cure cervical cancer through chemotherapy and radiotherapy surgery in general have not been able to provide

effective results. This resulted in many found alternative ways of treatment, among others, using materials from nature. One of the potential ingredients as anti-cancer based on some of the previous findings is Pedada leaves (Sonneratia caseolaris L.)

Pedada leaves are taken by plucking directly, taken leaves that have not yellowed, not consumed caterpillar, and is estimated to be old enough that expected content of active substances in the leaves is quite a lot. The samples that have been obtained are sorted, after which they are washed with running water. After that dried in a way aerated. After dry enough the samples are cut into small pieces to facilitate the drying process.After the sample is dry, then the sample will be extracted.

Extraction was performed by multilevel maceration method using n-hexane solvent, ethyl acetate, ethanol and water. The principle of the maseration method is the sifting of the active substance component of the simplicia by soaking the powder of the simplicia in the liquid of the dancer. The liquid of the dancer enters the cell's cavity through the cell wall and dissolves the active substance present in the cell. Because the difference in concentration of active substances inside and outside the cell causes the diffusion of the active substances present in the cell to exit the cell. And so on until there is equilibrium (Ditjen POM, 2000). This stratified massage aims to separate extracts between polar extract, semi-polar extract and non polar extract. After it is macerated, the sample is refluxed. The principle of the reflux method is the withdrawal of the chemical components carried out by means of samples inserted into the round bottom flask together with the liquid of the dancer then heated, the vapors of liquid dancers condensed on the condenser into liquid molecules of the dancer that will fall back toward the round bottom flask, and so on continuous until perfect sustenance, reflux for 3-4 hours (Ditjen POM, 2000). This reflux aims to draw the remnants of chemical components that have not been attracted by the prior solvent.

Simplified leaf pedada extracted as much as 600 grams. First, the simplia is macerated using n-hexane dancers for 3x24 hours. Afterwards the sample is aerated, aiming to vaporize the residual liquid of the container contained in the sample. The second meteration uses an ethyl acetate solvent for 3 × 24 hours, after which the sample is aerated again. The third maceration uses 96% ethanol solvent, after which the sample is aerated again. After diangin-aired, the sample then refluxed using aquades of aquades for 4 hours.

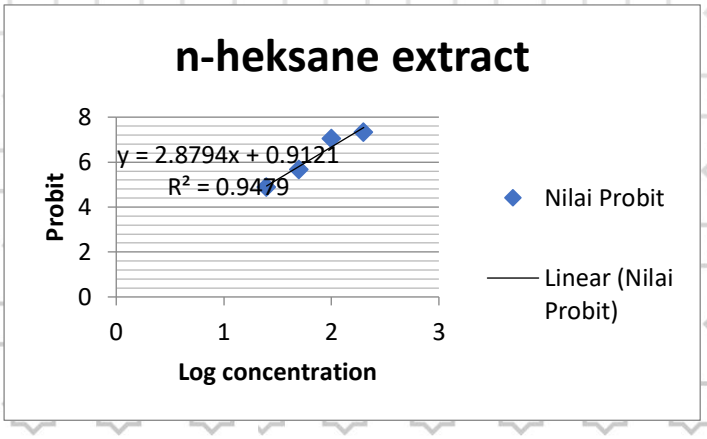
After obtaining n-hexane extract, ethyl acetate extract, and liquid ethanol extract then concentrated with the aid of Rotary evaporator. The principle of concentration of extract on this tool is by separating the extract with the liquid pengarinya based on the boiling point, so it will get a more concentrated extract or a more viscous extract. While the liquid water extract concentrated using water bath to obtain a more concentrated extract.

Hematotoxic Cell Test

The result of extraction of pedada leaf (Sonneratia caseolari L.), extract from leaf pedada obtained by each is n-hexan extract as much as 8.3 gram, etil acetate extract as much as 19,05 gram, ethanol extract 96% counted 24 gram, as much as 4.15 grams.

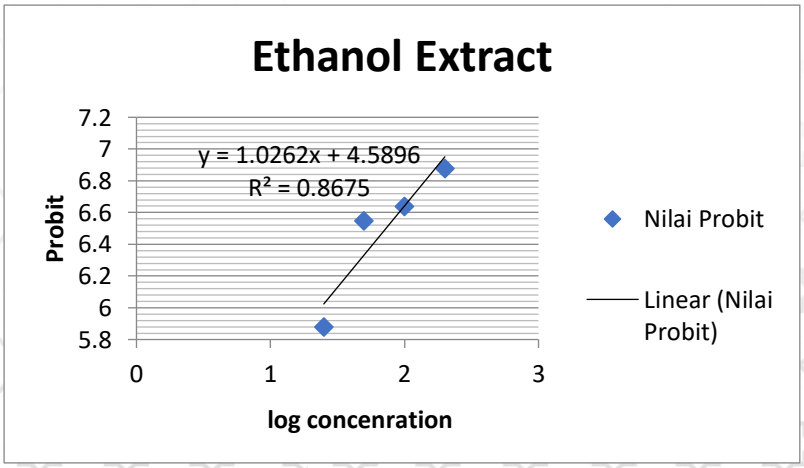
Results of Cytotoxic Test of MTT Cell Hell Method

n-heksane extract



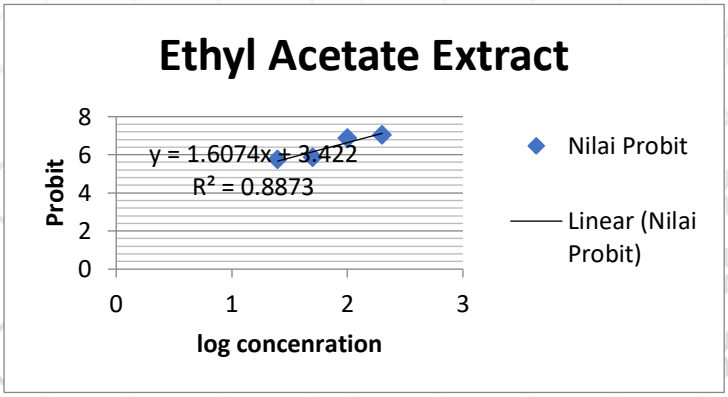
IC_{50} Calculation Result Curve of n-hexane Pedada extract (Sonneratia caseolaris L.)
According to the Probit Log-Concentration Graph Method

Ethanol Extract



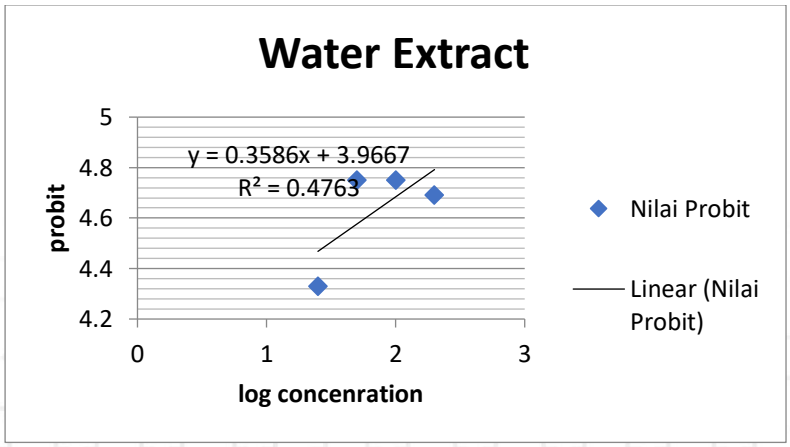
IC_{50} Calculation Result Curve Ethanol Pedada Extract (Sonneratia caseolaris L.)
According to the Probit Log-Concentration Graph Method

Ethyl Acetate Extract



IC50 Calculation Result Curve Extract Etil Acetate pedada (Sonneratia caseolaris L.)
According to the Probit Log Chart-Concentration Method

Water Extract



IC50 of Curve of Calculation Result of Pedada Water Extract (Sonneratia caseolaris L.)
According to Method of Probit Chart Log-Concentration

Next look for KLT profiles from 96% ethanol extract by using a certain eluen ratio. The principle of TLC is the displacement of the analyte in the stationary phase due to the influence of the mobile phase (Rohman, 2007). If the stain sightings have not been obtained or if the position of the stain is too upwards then elegance polarity is lowered or if the stain position is too below then eluent polarity needs to be improved. The extract is bottled on the plate, then the plates elute in a saturated chamber. The saturation of the chamber is intended for the elution process of the eluent to be derived only from the eluent of the chamber base and not from the

yawning eluent if the chamber is unsaturated. After that the plates are inserted in a saturated chamber.

After the plates are eluted, the plates are removed from the chamber and then allowed to dry. Subsequently the stains on the plate were observed under a 254nm UV lamp, 366nm then sprayed with cerium sulfate and heated over the heater. At 254 nm UV, the plate will fluctuate while the sample will appear dark in color. The appearance of stains on 254 nm UV lamps is due to the interaction power between UV rays and the fluorescence indicator found on the plates. In UV 366 nm the stain will flouresensi and the plate will be dark. The appearance of stains on 366 nm UV lamps is due to the interaction power between UV light and the chromophore group bound by the auxochrome present in the stain. So that the visible stains on UV 366 lamps look bright because the silica gel used does not fluororesensi on 366 nm UV rays. While the appearance of a stain by serium sulfate is because it is a reductor so it can break the double bond that will cause the shift wavelength to a longer direction so it can be seen by the eye.

The result of identification of TLC of ethanol extract of pedada leaf (Sonneratia caseolaris), on the appearance of 254 nm UV stain was not obtained stain. While at 366 nm UV obtained 5 stain its Rf value is 0.84; 0.75; 0.62; 0.51 and 0.22. The Rf value can be defined as the distance traveled by the solvent from the origin. Therefore the Rf number is always smaller than 1.0. The price of Rf lies between 0.2-0.8 to maximize the separation.

Furthermore, the fractionation by using column chromatography (KK). The fractionated ethanol extract is 15 grams, using silica gel 60PF254 stationary phase and the mobile phase with an increasingly polar gradient ie hexane: (ethyl acetate: (10: 1), (7: 1), (5: 1), (3: 1), (1: 1)}, chloroform, chloroform: methanol {(10: 1), (5: 1), (3: 1), (1: 1)}, methanol. The fractionation results were then analyzed using thin layer chromatography (TLC) method. This is done with the aim of further grouping the fractions obtained based on the similarity of the chemical content profile of the TLC spots formed.

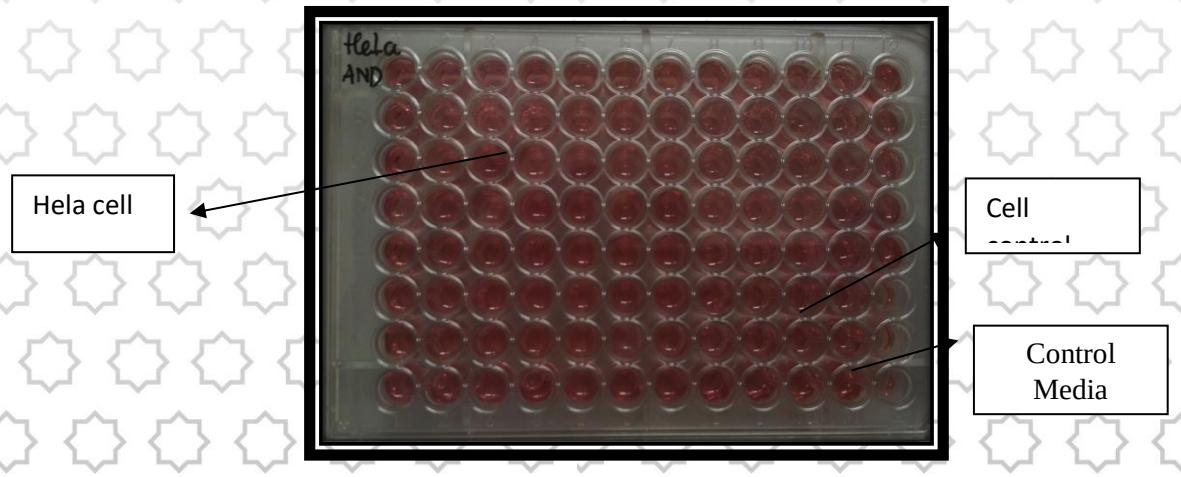
The result of fractionation of ethanol extract of pedada leaf (Seonneratia caseolaris), obtained 12 fractions. the most active fraction has been combined, subsequently re-chromatographed the 2nd column to obtain a single compound or pure compound.

This study was conducted to determine the toxicity of n-hexane extract, ethanol extract, ethyl acetate extract, water extract and pedada leaf isolate and determine the value of IC50, which is the concentration of a compound that can inhibit cell growth by 50%.

Cytotoxic tests of cancer cells are a common baseline test on anticancer drugs and chemo-revealing compounds. Through IC_{50} parameters, it can be seen the toxic potential of the tested

compounds / materials. One of the common methods used for in vitro cytotoxicity testing is the MTT method. The cells used in this study were cell hela.

Cytotoxic test by MTT method



Cell Condition Draw Before treatment

Pictures After the treatment of the test

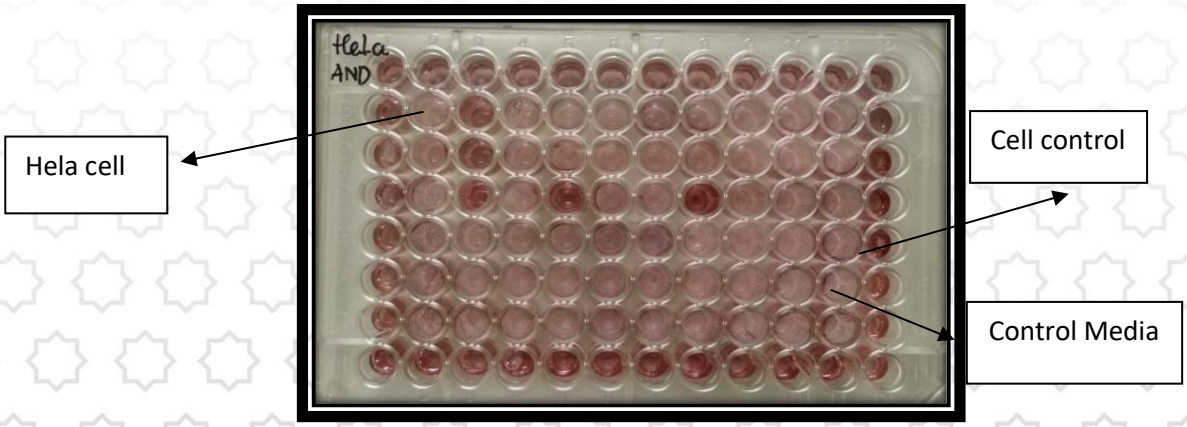
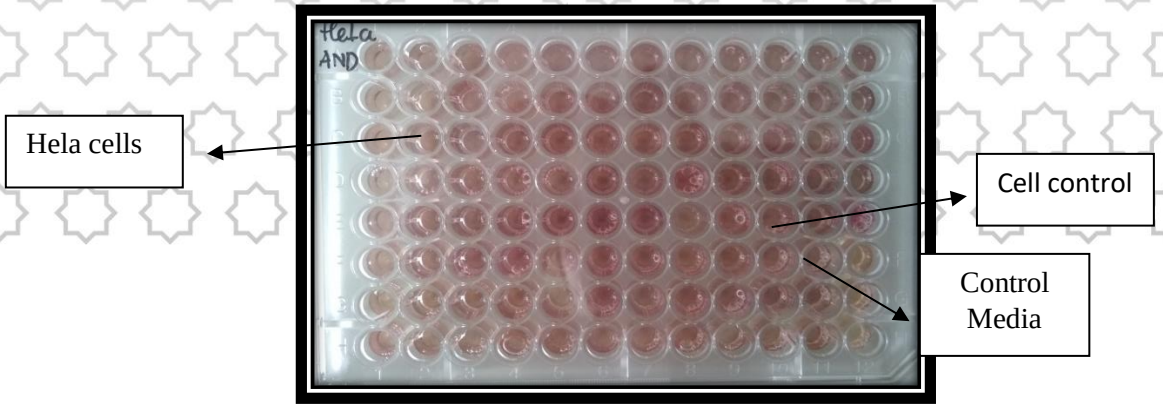


Figure After MTT reagent and incubated 24 hours



Picture After Granting of SDS and has been incubated 24 hours

Hela cells before and after treatment

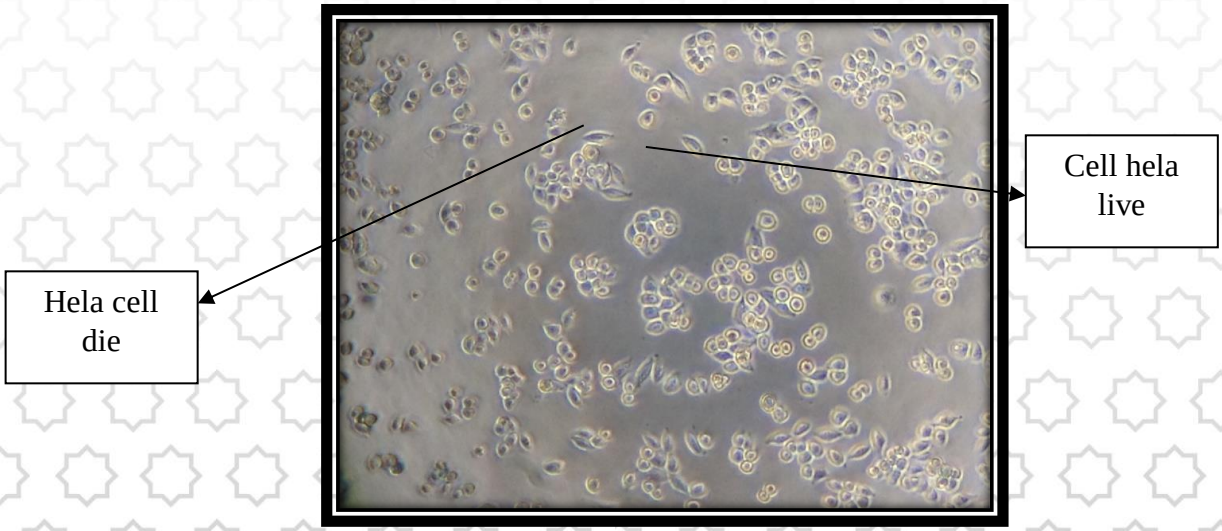


Figure of Hela Cells Before Treatmentel Helat

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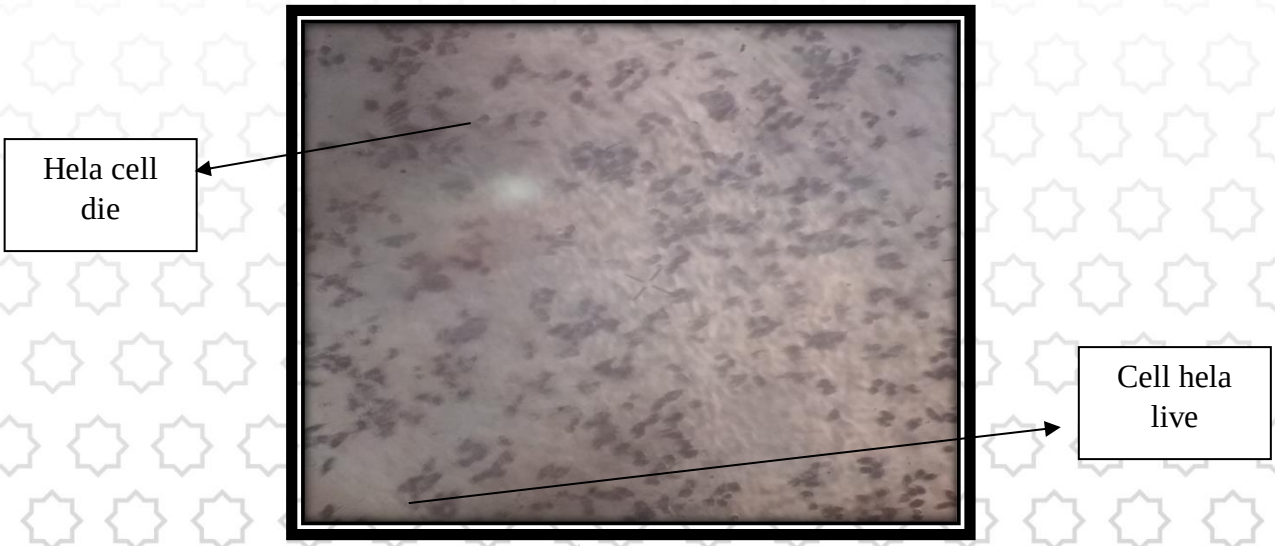


Figure Treatment Results N-hexane extract at 200 µg / ml concentration

Draw Treatment Results of ethyl acetate extract at a concentration of 200 µg / ml

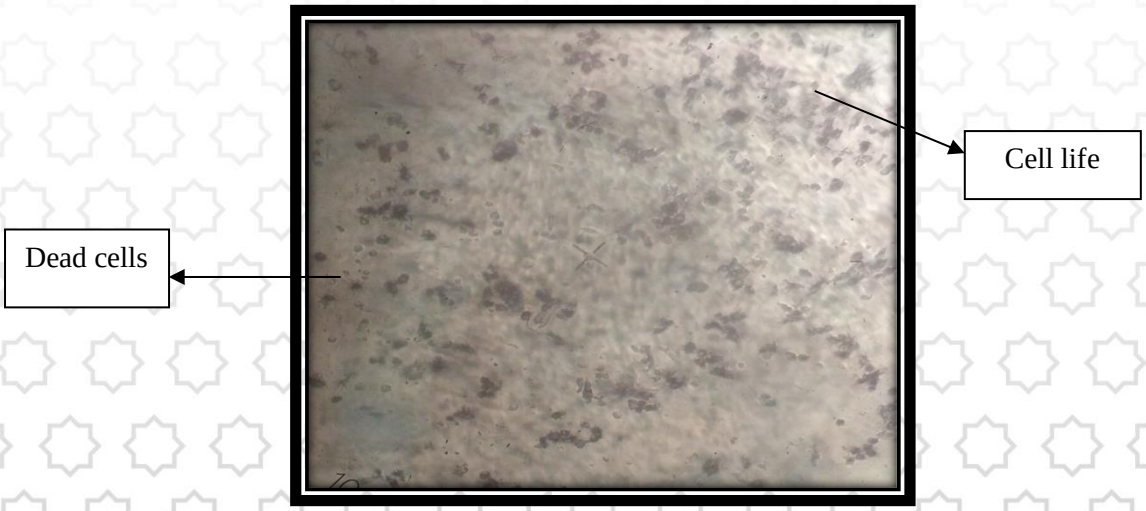


Figure Results of treatment of ethanol extract at a concentration of 200 µg / ml

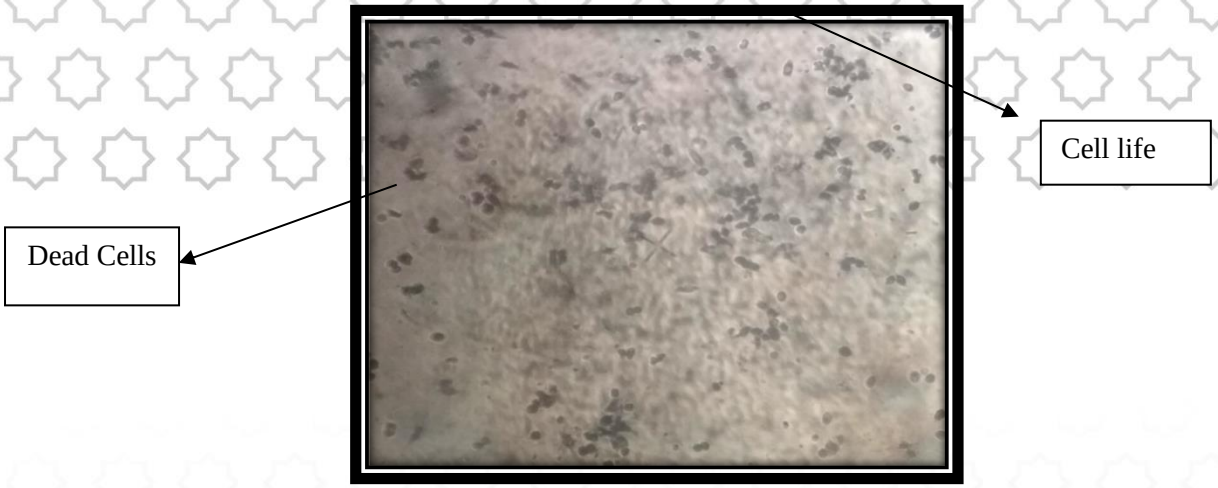


Figure Results Treatment of water extract at a concentration of 200 µg / ml

For hela cell test, initially extract and pedal leaf isolate were weighed as much as 10 mg and dissolved using DMSO solvent as much as 200 µl, so obtained stock solution with concentration 50.000 µg / ml. In this test the DMSO solvent is used because it is a good solvent for inorganic ions and organic compounds. DMSO itself has cytotoxic properties at certain concentrations but at low concentrations the DMSO has relatively no effect on cell growth. The use of DMSO as a solvent in various concentrations is relatively unaffected in cell viability T47D (Nurulita, 2005). This indicates that in this study, cell death or cell growth inhibition was not due to DMSO effect but because of the influence of test samples used in the study. The test solution was prepared with a concentration of 25; 50; 100; 200 µg / ml.

Cell harvest is a procedure in which cells that have been grown in a medium containing 10% Fetal Bovine Serum (FBS) as a source of nutrients, penicillin-streptomycin 0.25% as anti-

bacterial, and DMEM media and incubated in incubators at 37 ° C with flow 5% CO2, transferred to conical tube. Thus, when the cells have been confluent (cells that have grown and can be harvested) are then removed from the tissue culture plate by tripsination technique, ie cells washed with PBS 2-3 times to remove the growth media previously present in the plate because one of the cell content is harvested ie FBS can stop trypsin work. Tripsin 0.025% of 1 ml is added to release the cells attached to the plate. For 10 minutes it is left in the incubator to maximize the action of trypsin, then observed the loose cell under a microscope. The loose cells were transferred into a 15 ml conical tube tube, added 5 ml of complete medium (FBS 10%, 0.25% sterilizer and DMEM media) to provide nutrients for the cells and to avoid contaminants. Taken 10 µl inserted into the haemocytometer to determine the number of cells present in 1 ml. Cell counting during harvesting is done using a haemocytometer and viewed under a microscope. Healthy cells are characterized by cell-shaped round, berinti, protected by a clear cell wall and glowing under a microscope. While the dead cells look dark with a damaged cell nucleus. The number of cells used is 5 x 103 cells per well. The total number of cells required to plant 5 x 103 cells is 5x105 cells, each well would fill 100 µl of complete media containing cells, then the total volume required to plant cells is 10 ml. The cell suspension in a complete medium of 100 µl was added to the microplate 96 wells to be grown and then incubated for 24 hours in order for the cell to adapt and stick to the bottom of the plate. After incubation, a test solution of each extract at 25 µg / ml was added; 50 µg / ml; 100 µg / ml; 200 µg / ml each of 100 µL with 3 replications. The complete media of 100 µL was added to the other 4 wells containing the cell as cell control and 3 wells were left blank as incubation at 37 ° C in a 5% CO2 incubator for 24 hours to determine the effect of the compound on the inhibition of cell division. After 24 hours incubation, it appears that many WiDr cell deaths were due to the treatment of extract samples and pedada leaf isolates and can be seen from the morphological changes in the cells. The observations under living cell microscope appear to be attached to the bottom of the plate and are brightly colored, while the dead cells are released from the bottom of the plate and are dark in color.

Subsequently at each well, 100 µL of MTT solution was added, in which MTT would be absorbed into living cells and broken down by the reduction reaction by the reductase enzyme in the mitochondrial respiration chain to formazan. Cells were incubated for 4 hours in a 5% CO2 incubator temperature of 37 ° C to maximize MTT work.

MTT is a reagent used for the purpose of facilitating observations in living cell counting. The MTT reagent is a water-soluble tetrazolium salt by producing a yellow solution. The basic principle is the work of mitochondrial enzymes in active cells that metabolize the salt of tetrazolium, resulting in the termination of the tetrazolium ring by the dehydrogenase enzyme causing the tetrazolium to turn into a water insoluble formazan but soluble in 10% and purple (10MM, 1983) SDS.

The intensity of this purple color has a direct correlation with the number of living cells. While dead cells will not be affected by MTT reagents because the mitochondrias do not resonate so that the tetrazolium ring is not interrupted it will not form a purple formazan, but the color remains yellow.

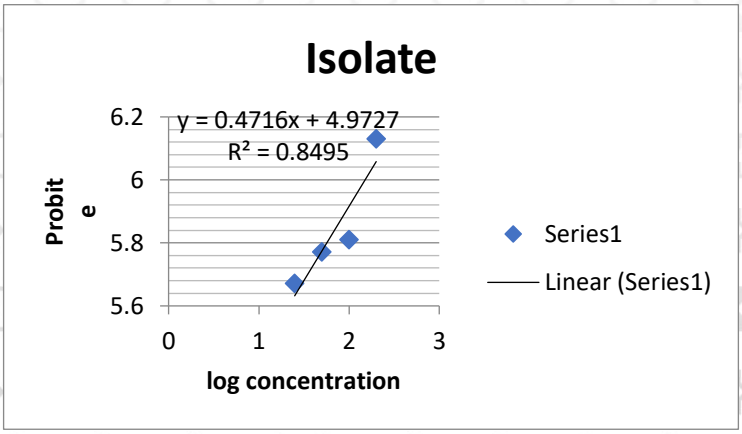
The MTT reaction was discontinued by adding 50 µL SDS to break the formazan crystals formed and to provide a purple color, incubated in room temperature with a paper-covered state that covered the entire surface of the microplate overnight to maximize the action of the stopper reagent and prevent the oxidation of MTT in the presence of light. The cessation of reactions between MTT reagents and living cells is by the addition of 10% SDS in 0.1 N HCl which can denature the protein into polypeptide units and form the SDS polypeptide complex. SDS 10% can dissolve formazan crystals from the reaction of MTT and does not cause precipitation. SDS is used as much as 10% because the formazan crystals are not completely soluble if SDS is less than 5% and readily dissolves at 37 ° C (Tada et al., 1986).

The uptake is read with a microplate ELISA reader at a 595 nm wavelength. Used wavelength 595 nm because at that wavelength is obtained optimum measurement so that will get sensitive and specific data. The stronger the intensity of the purple color that is formed, the greater the absorbance will be obtained. This suggests that the more living cells react with the tetrazolium salt, formazan is formed too much.

In the MTT method, the percentage of cell death was the difference in absorbance of control by absorbance of the sample divided by 100% controlled absorbance. The data is processed by using Probit analysis to obtain IC50 sample value. IC50 value shows the percentage of cell death in culture as much as 50%.

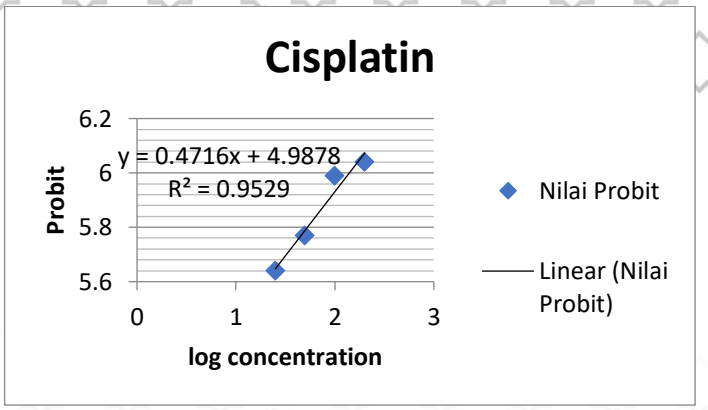
The results obtained on the test using hela cell culture for leaf n-hexane leaf extract 'with concentration of 25 µg / ml; 50 µg / ml; 100 µg / ml; 200 µg / ml with the percentage value of inhibition as follows: 46,2051%; 75.6209%; 98.1600%; 99.0800%. extract of ethyl acetate leaves pedada 'with concentration 25 µg / ml; 50 µg / ml; 100µg / ml; 200 µg / ml with the percentage value of inhibition as follows: 78,1968%; 81.4167%; 97.7920%; 98.8040%. ethanol extract of pedada leaves' with concentration 25 µg / ml; 50 µg / ml; 100µg / ml; 200µg / ml with the percentage value of inhibition as follows: 81,6007%; 94.6642%; 95.9521%; 97.6080%. water extract of pedada leaves with concentration 25 µg / ml; 50 µg / ml; 100 µg / ml; 200 µg / ml with the percentage value of inhibition as follows: 25,4829%; 30.0827%; 30.3587%; 38.5464%.

Isolate



IC_{50} Calculation Result Curve Isolate pedala (Sonneratia caseolaris L.)
According to the Probit Log-Concentration Graph Method

Cisplatin

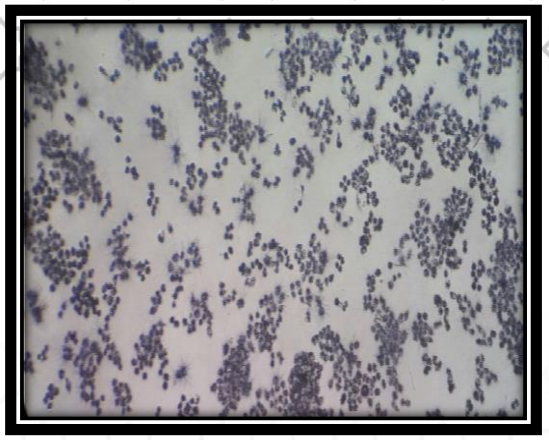


IC_{50} Calculation Result Curve of Cisplatin extract According

to Probit Log-Concentration Graph Method

Figure Results of treatment of isolates at a concentration of 200 µg / ml

The negative control hela cells



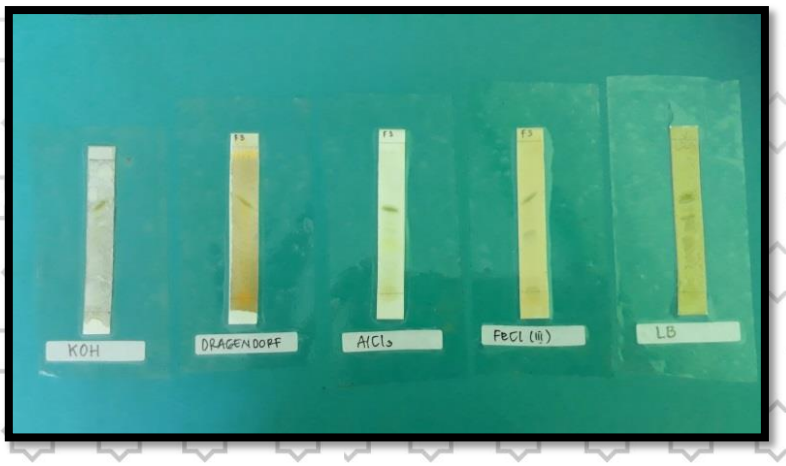
Picture of Cell Control

Isolate of Pedada leaves with concentration 25 µg / ml; 50 µg / ml; 100 µg / ml; 200 µg / ml with the percentage value of inhibition as follows: 75,9889%; 78.3808%; 79.9448%; 87.9484%. Cisplatin with a concentration of 25 µg / ml; 50 µg / ml; 100 µg / ml; 200 µg / ml with the percentage value of inhibition as follows: 74.701%; 78.1968%; 79.9448%; 87.9484%.

Based on the data processing that has been done with probit analysis method, IC50 value for hela cells from the test using n-hexan leaves leaf extract of 26.28 µg / ml; extract of ethyl acetate leaves pedada 9,58 µg / ml; ethanol extract of pedada leaves 2.51 µg / ml; water extract of pedada leaves 760,32 µg / ml; 1.14 µg / ml leaflet isolates and 1.06 µg / ml cisplatin.

Determination of the toxic limit of this study, according to (Mans, 2000) mentions an extract is active or positively menghabat cell growth in the proliferation test as an anticancer agent if

it has IC50 ≤ 50 µg / ml. According to national cancer institute (NCI), pure compounds classified as active inhibiting the proliferation of cells when the value of IC50 ≤4 µg / ml. These results suggest that n-hexane, ethanol, ethyl, and pedada leaf isolates have a cytotoxicity effect on hela cells and thus have potential as anti-cancer agents. However, the water extract has no effect as an anticancer against HeLa cells but is specific to certain cancer cells, but can also be caused by errors during the work that can be contaminants, thus less potential for hela cells because the value of IC50 obtained is greater than 50 µg / ml.



Identification of classes of compounds

CONCLUSION

Based on the research that has been done can be concluded that;

1. The ethanol extract, ethyl acetate, n-hexane, and pedada leaf isolate (Sonneratia caseolaris L.) have cytotoxic activity against cervical cancer cells, while the water extract is less active in inhibiting Hela cell growth.
2. Leaf pedada (Sonneratia caseolaris L.) has potential as a natural anticancer material because, from the research results of ethanol extract, ethyl acetate, n-hexane, and pedal leaf isolate (Sonneratia caseolaris) have cytotoxic activity with IC50 values as follows 2.51 µg / ml; 9.58 µg / ml, 26.28 µg / ml, 1.14 µg / ml, while the water extract is less potent against hela cells with IC50 760,32 µg / ml.

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The Correlation Between Cigarette Smoking and Hearing Loss

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Abstract

Introduction: Tobacco consumption has become a common tendency worldwide, while smoking is a well-known risk factor for many health problems including hearing loss both sensorineural hearing loss and conductive hearing loss. Consumed tobacco may affect cochlear blood supply by increased blood viscosity, reduced available oxygen and cause histopathological changes in the middle ear lining tract. The aimed of this study was to determine the correlation between cigarette smoking and hearing loss.

Method: We enrolled 38 participants aged 20-85 years old that conducted pure tone audiometric testing at PKU Muhammadiyah hospital during November 2017-Januari 2018 and frequencies tested were 0.5, 1, 2, 3, 4, 6, and 8 kHz. Smoking status was categorized into two groups; mild smoking and heavy smoking group.

Result: There are 38 subject research, 8 of them (13, 2%) are categorized as mild deaf, 11 of them (23, 7%) are categorized as moderate deaf, and 19 of them (63, 2%) are categorized as severe deaf. Based on their smoking activity, 17 of them (44, 7%) are light smokers and 21 of them (55, 4%) are heavy smokers. There is no statistically significant correlation between smoking activity and the hearing degree (r -0.085, p 0.778), with the p value varied in each frequency.

Conclusion: There is no correlation between smoking activity and the degree of hearing loss, because there are many other factors besides smoking that could decrease the degree of hearing loss, such as age and medical chart of comorbidity.

Keywords : Smoking, Hearing Loss, Tobacco, Risk Factor

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INTRODUCTION

Tobacco consumption or smoking is a common habit worldwide. In general, tobacco is consumed by approximately 1.3 billion of the world population [1]. Data from Indonesian Ministry of Health shows that there is an increase in the prevalence of smokers, from 27% in 1995 to 36,3% in 2013 [2]. Tobacco-related diseases are also increasing including associated with the development of hearing loss.

Nicotine and other toxic substances contained in cigarettes cause histopathological changes in the respiratory lining track and the middle ear, it is hypothesized that there could be a relationship between cigarette smoking and middle ear impairment, that result in persistent middle ear infections and hearing loss [3], [4].

Tobacco may also affect cochlear blood supply because it causes peripheral vascular changes, such as increased blood viscosity, press the vessels and the restriction process reduced oxygen availability. Smokers with normal hearing were found to have significantly reduced DPOAEs when compared to their normal counterparts [5], [6].

Smoking is a well-known risk factor for many health problems but the association between cigarette smoking and hearing loss has been inconsistent [7]. A previous study has shown that smoking is correlated with hearing loss in the geriatric population [8]. Other studies found that smoking pack-years and ageing have multiplicative effects on developing hearing impairment [9]. In this study, we aimed to determine the effect of smoking on the prevalence of hearing impairment among the general population.

MATERIALS AND METHOD

This research is quantitative research that uses an analytical descriptive method with cross-sectional approach to knowing the correlation between smoking activity and the hearing impairment among patients in ENT polyclinic PKU Muhammadiyah hospital. There are 38 subject examined pure tone audiometric during November 2017-Januari 2018 with the complaint of hearing loss. The inclusion criteria are: patients who have smoking behaviour, patiens who are >20 years old, male patients, and the exclusion criteria are: patients with congenital deaf and patients who work under noise exposure. Instruments used in this research cover questionnaire, medical record, and informed consent sheet. Analysis of bivariate data that has been obtained will be processed using Statistical Program for Social Science 23.0 (SPSS 23.0) for Windows and analysis of primary data uses Gamma Correlation Test.

RESULT AND DISCUSSION

Characteristics of the Subject

There are 38 subject according to ISO, 8 of them (13,2%) are categorized as of mild deaf, 11 of them (23,7%) are categorized as moderate deaf, and 19 of them (63,2%) are categorized as severe deaf. Among 38 participants analyzed, 17 (44.7%) were light smokers, 21 (55.4%) were

heavy smokers. Of the participants, 7 (18.4%) were aged between 20 and 35, 14 (36.8%) were aged between 36 and 55, 17 (44.7%) were aged between 56 and 85. The comorbidity of the subject were diabetes mellitus 10.6 % and hypertension 28.9 % (table1).

Table 1. Data of Respondent's Characteristics

Subject Variable		Percentage (%)
Average of Patients' Age (Mean ± SD)	Year	2,26 ± 0,760
Age	20 – 35	18,4
	36 – 55	36,8
	56 – 85	44,7
Comorbidity (Mean ± SD)	Disease	1,50 ± 0,688
	None	60,5
	Hypertension	28,9
	Diabetes Mellitus	10,5
Average of Degree of hearing loss (Mean ± SD)	Degree of hearing loss	2,50 ± 0,726
	Mild	13,2
	Moderate	23,7
	Heavy	63,2
Average of Smoking Status (Mean ± SD)	Status	1,55 ± 0,504
	Light	44,7
	Heavy	55,3

Based on audiometric results, diagnose on right ear shows that there are 5 normal patients (11,1%), 3 patients with mild SNHL (6,7%), 9 patients moderate SNHL (20,0%), 8 patients with moderate-severe SNHL (17,8%), 2 patients mild CHL and severe CHL (4,4%), 8 mixed patients (17,8%), and 1 patient with total deafness (2,2%) (table 2).

Table 2. The Distribution of Audiometric results on Right Ear

Diagnose	Number	Percentage (%)
Normal	5	11.1
Mild SNHL	3	6.7
Moderate SNHL	9	20.0

Moderate-Severe SNHL	8	17.8
Mild CHL	2	4.4
Severe CHL	2	4.4
Mixed HL	8	17.8
Total Deafness	1	2.2

Diagnose on left ear shows that there are 9 normal patients (20,0%), 5 patients with mild SNHL (11,1%), 6 patients with moderate SNHL (13,3%), 10 patients with Moderate-severe SNHL (22,2%), 1 patient with mild CHL (2,2%), 5 mixed patients (11,1%), and 2 patients with total deafness (4,4%) (table 3).

Table 3. The Distribution of Audiometric results on Left Ear

Diagnose	Number	Percentage (%)
Normal	9	20.0
Mild SNHL	5	11.1
Moderate SNHL	6	13.3
Moderate-Severe SNHL	10	22.2
Mild CHL	1	2.2
Severe CHL	0	0
Mixed HL	5	11.1
Total Deafness	2	4.4

The correlation between smoking activity and the hearing impairment among patients in PKU Muhammadiyah Hospital is examined by using Gamma correlation test.

Table 4. The Correlation between Smoking Activity and Degree of Hearing Loss.

	Hearing Loss			Coefficient correlation	p
	Mild	Moderate	Severe		
Light Smokers	4 (10.5%)	1 (02.6 %)	12 (31.6%)	-0.085	0.778
Heavy smokers	1 (02.6 %)	8 (21.0%)	12 (31.6%)		

5 (13.1%)	9 (23.6%)	24 (63.2%)

The correlation between smoking activity and degree of hearing loss in patients that performed pure tone audiometric at PKU Muhammadiyah Hospital is examined using gamma correlation test. Table 4 shows the result of the statistic test on research subject whose smoking status is light and heavy. The result is there are 4 light smokers (10.5%) suffer from mild hearing loss, 1 light smoker (2.6%) suffer from moderate hearing loss, 12 light smokers (31.6%) suffer from severe hearing loss. Meanwhile, 1 heavy smoker (2.6%) suffer from mild hearing loss, 8 heavy smokers (21.0 %) suffer from moderate hearing loss, and 12 heavy smokers (31.6%) suffer from severe hearing loss. The *p* value is 0,778, which means there is no correlation between smoking status and degree of hearing loss in PKU Muhammadiyah Hospital and the coefficient correlation shows a strong downhill (negative) linear relationship.

This result differs from Sung et al., 2013 that report the current smoking significantly influenced hearing loss at all frequencies in workers exposed to occupational noise, and heavier smoking influenced low-frequency hearing loss more greatly. There was a dose–response relationship between smoking amount and low-frequency hearing thresholds; however, this was not observed for high-frequency hearing thresholds. [10] Another study report that Current smoking was associated with hearing impairment in both speech-relevant frequency and high frequency across all ages. However, except in the ages of 40s, passive smoking was not related to hearing impairment in either speech-relevant or high frequencies [11].

The higher threshold of hearing value in dB means the more severe the degree of hearing loss. This study proves that smoking has no significant risk in decrease in threshold of hearing. We can prove it by looking at the *p* value using Gamma correlation test analysis. The goal of this test is to see whether the correlation of threshold of hearing in each frequency has significant result toward smoking status. First, the normality of data is examined and the result is smaller than 0,05, which means the data is not distributed normally. The result from each frequency is varied, but the result that is closest to 0,05 is p=0,243. It could be caused by the influence of age distribution since the research subject is dominated by those who are 56-85 years old (44,7%). As Ahmed Faisal, et al (2015) argued in his study entitled *Cigarette Smoking causes Hearing Impairment in Bangladeshi Population*, the prevalence of hearing disorder has a bigger percentage in older smokers (>40 years old). Smoking causes hearing disorder in young smokers either, but its percentage is relatively lower [5].

Based on the data from audiometry results, which has been processed by Gamma correlation test analysis, the *p*-value = 0,778, which means there is no significant relationship between smoking status and degree of hearing loss. It is because there are many factors besides smoking that cause a hearing disorder. In this research, the subject research is mostly diagnosed as sufferers of SNHL (Sensorineural Hearing Loss). Its percentage is 20,0% on right ear and 22,0% on left ear. Based on a study conducted by Justin K. Chau et al. entitled *Systematic Review of the Evidence for the Etiology of Adult Sudden Sensorineural Hearing Loss,* it is stated that the etiology that cause SNHL are idiopathic (71,0%), infectious diseases (12,8%),

othologic diseases (4,7%), trauma (4,2%), vascular or hemathology (2,8%), neoplastic (2,3%), and other factors (2,2%) [12].

Hearing impairment is affected by various social factors, such as smoking, alcohol use, occupational or leisure noise exposure, and occupational vibration, as well as different medical factors, such as hypertension, diabetes, and increased serum cholesterols. [13,14] Some factors that could lead to hearing disorder in this study are hypertension and diabetes mellitus. Table 1 shows that 28.9% of subject research have a medical chart of hypertension and 10.5% of them have a medical chart of diabetes mellitus. It is mentioned that hypertension is a factor that accelerates degeneration of the organ of hearing, which is related with the aging process.

CONCLUSION

There is no correlation between smoking activity and the degree of hearing loss, because there are many other factors besides smoking that could decrease the degree of hearing loss, such as age and medical chart of comorbidity.

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Phytochemical Screening and In Vitro Antidiarrheal Activity of Calingan (*Rubus rosifolius*) Leaves Extract

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Abstract

Calingan (*Rubus rosifolius*) leaves are used by Tengger tribe in Probolinggo District, East Java to treat diarrhea. The aim of this study was to determine phytochemical content and antidiarrheal activity of *R. rosifolius* leaves extract. Maceration method in ethanol 96% was performed for extraction. Preliminary phytochemical screening was carried-out by using tube test. The study showed that ethanolic extract of *R. rosifolius* contained saponins, polyphenols, tannins, and flavonoids. In vitro antidiarrheal activity test was performed by using wells diffusion method with various concentrations, i.e. 160; 320; 640; 1280 mg/mL. The in vitro test was compared with ampicillin and gentamicin as positive controls. The result was analyzed by one way analysis of varian test with significance level in 95%, followed by Post Hoc test. The results showed that ethanolic extract of *R. rosifolius* leaves inhibited the growth of *Staphylococcus aureus* and *Eschericia coli* at concentration of 1280 mg/mL with diameters of inhibition zone of 9.200 ± 0.264 and 14.500 ± 1.323 mm. Meanwhile, the positive controls at concentration of 20 µg/mL inhibited the growth of *S. aureus* and *E. coli* with diameters of inhibition zone of 25.000 ± 1.000 and 15.167 ± 2.021 mm, but the controls did not inhibited the growth of both bacteria. Based on the study, the phytochemicals may contribute to antidiarrheal effect of *R. rosifolius* leaves extract.

Keywords : *Rubus Rosifolius*, Tengger, Phytochemical Screening, Antidiarrheal Activity

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INTRODUCTION

Diarrhea is a disease that still becomes one of the main causes of death for children under 5 years old (Saeed et al., 2015). In general, diarrhea is an infection symptom in the digestive tract which can be caused by various bacteria, viruses and parasites of the organism. In Indonesia, diarrhea is an endemic disease that is highly potential for Extraordinary Events or Kejadian Luar Biasa (KLB) which end up in death and high Case Fatality Ratio (CFR). The percentage of CFR for diarrhea disease in 2016 was 3.04% (Indonesian Ministry of Health, 2017). When it was viewed from the age group, the highest diarrhea incident was recorded in children aged <1 year old (5.5%) , while at the age of 1-4 years old the diarrhea incident was 5.1% (Indonesian Ministry of Health, 2016). Death cases from diarrhea disease were caused by the dehydration which results in around 1.8 million deaths each year (Ahs et al., 2010).

One of the handling efforts of diarrhea is by replacing the lost body fluids. The first recommendation in handling dehydration due to diarrhea is Low-osmolarity oral rehydration solution (ORS). Other diarrhea treatment is by using antidiarrheal medication which is loperamid as an antimotility that can be used by adults and children (Faure, 2013). Although the use of loperamid in adult patients has shown some efficacy in several studies, its uses in children has been reduced by the World Health Organization (WHO) and the American Academy of Pediatrics because of the worried about the efficacy and safety in young children. It becomes something that needs to be reconsidered, especially in diarrhea cases in children (Faure, 2013). This led to the need of new medicine from natural ingredients.

R. rosifolius is one of the plants from Tengger tribe of Probolinggo Regency, East Java which has the efficacy to cure diarrhea. According to Scio et al. (2012), R. rosifolius leaves contained alkaloids, sterols, tannins, saponins and flavonoids. Those compounds ware suspected to be responsible for antidiarrheal activity. Therefore, it is important to determine the antidiarrheal activity of *R. rosifolius* leaves that growth at Tengger tribe in Probolinggo District, East Java through in vitro aasay including the determination of other secondary metabolites.

MATERIALS AND METHOD

This research was conducted at Phytochemical Laboratory and Microbiology Laboratory of Faculty of Pharmacy, University of Jember in December 2017 - January 2018. The tools used in this research were incubator, autoclave, refrigerator, rotary evaporator, spectrophotometry, and laminar air flow. The main material used in this research was *R. rosifolius* leaves that were identified by Ujang Tri Cahyono, SP.MM at the Plant Laboratory of Jember State Polytechnic with voucher number of 012/PL17.3.1.02/LL/2018. The other materials were 96% ethanol, dragendorf reagent, wagner reagent, mayer reagent, H_2SO_4 2N, concentrated HCl, concentrated H_2SO_4, sterile aquadest, 1% BaCl, H_2SO_4, 0.9% NaCl, Nutrient agar (NA), MHA, Ampicilin, Gentamicin, and DMSO. The isolates of *S. aureus* and *E. coli* bacterias were obtained from Microbiology Laboratory of Faculty of Pharmacy, University of Jember.

Preparation of Extracts

350 grams of dried *R. rosifolius* leaves were macerated with 1750 mL 96% ethanol, covered with aluminum foil and left for 5 days at room temperature and stirred occasionally, then filtered to produce filtrate. Residue were remacerated with the same method. The filtrate was evaporated with a vaccum rotary evaporator at 200 rpm and a temperature of 50°C until a thick extract was obtained.

Phytochemical Screening

Alkaloids Identification

0.3 grams of extract was added with 5 mL of HCl 2N, heated for 2-3 minutes. After being cool, it was added with 0.3 grams NaCl, stirred evenly then filtered. Then, the filtrate was added with 2 N HCl and wagner reagent. The test result was stated to be positive if there was turbidity or sediment.

Saponin Identification

A total of 0.3 grams of extract in 10 mL of aquadest were shaken for 30 seconds. The test result was positive if the foam was formed for 30 minutes with a height of 3 cm above the surface.

Triterpenoids identification

The extract was mixed with chloroform and filtered. The filtrate was given a few drops of acetate, heated, cooled, and then added with sulfuric acid. The test result was stated positive if red sediment was formed.

Steroids Identification

Sterols and steroids were tested using Lieberman reaction. 10 mL of ethanol extract was evaporated and the residue was mixed with 0.5 mL of hot acetic anhydride and 0.5 mL of chloroform. The mixture was tested using Lieberman Burchard reagent. The test result was positive if a blue-green ring was formed.

Polyphenols and Tannins Identification

0.3 grams of extract in 10 mL of hot aquadest was stirred and left to room temperature. The mixture was added with 3-4 drops of 10% NaCl, stirred, and filtered. The filtrate is divided into 3 parts, called IIA, IIB, and IIC. IIA solution as blank.

$FeCl_3$ assay

A few drops of $FeCl_3$ solution were added to IIB solution. Formation of a blackish green color indicated the presence of tannin. Then, the mixture was added with gelatine and NaCl. If there was no precipitation, it was added with a few drops of $FeCl_3$ solution. A color change into blue green or black revealed the presence of polyphenols.

Gelatin assay

A little amount of 1% gelatine solution and 5 mL of 10% NaCl solution were added to IIC solution. A white precipitation indicated the presence of tannins.

Anthraquinone identification

0.3 grams of extract was extracted with 10 mL of distilled water, and filtered. The filtrate was extracted with 3 mL of toluene in a separating funnel. The toluene phase was collected, added with ammonia and shaken. The test result was positive if it showed a red color.

Flavonoids Identification

0.3 grams of extract was shaken with n-hexane until it was colorless then added with ethanol. The test result was positive for leucoanthocyanins if it was added with concentrated HCl and heated; its color changed into bright red color. The test result was positive for flavones if it was added with concentrated HCl and magnesium; it turned into red. The test result was stated to be positive for flavonol if concentrated HCl and magnesium were added, then it turned to pale red. The test result was positive for flavonones if concentrated HCl and magnesium were added, it turned dark red.

In vitro antidiarrheal activity test using E. coli and S. aureus

The method used in this research was the well diffusion method based on Nayak and Lazar's (2014) with a little modification. 15 mL of sterile MHA was poured into a sterile petri dish in aseptic condition, until it condensed. The media was inoculated with bacteria using a cotton swab. Each well was filled with 20 µL of test solution with different concentrations, positive control and negative control, then incubated at 37±2 °C for 24 hours. The observation of the formation of inhibitory zone in the media was performed after 24 hours. The inhibitory zone was calculated by measuring the diameter of the inhibition around the well (in mm) using the calipers which was reduced by the diameter of the well.

Data analysis

All measurements were taken in three times replication, and the result obtained were expressed as mean ± SD. The comparison between the dependent variables were determined using the analysis variance (ANOVA) by the software SPSS 16.0 with significance level in 95%, followed by Post Hoc test.

RESULT AND DISCUSSION

This research was conducted on *R. rosifolius* leaves to determine the presence of secondary metabolites. Secondary metabolites of *R. rosifolius* leaves had been analyzed qualitatively and the results were presented in Table 1. The phytochemical screening showed that flavonoids, tannins, and saponins gave positive results, while alkaloids, triterpenes, steroids and anthraquinones gave negative results. According to Scio et al. (2012), *R. rosifolius* leaves contained several secondary metabolites including alkaloids, tannins, flavonoids, and saponins. The absence of alkaloids compounds in *R. rosifolius* leaves may be due to their low concentration, so as to give negative results (Scio, 2009) or may be because of differences of location for plants growth.

Table 1. Phytochemical screening of *R. rosifolius* leaves extract

Phytochemical content	Method	Result
Alkaloids	Wagner test	Negative
Saponin	Foam test	Positive
Triterpenoid	Liebermann test	Negative
Steroids	Liebermann test	Negative
Polyphenols	Ferricloride test	Negative
Tanin	Ferricloride test	Positive
Antrakinon	Borntrager test	Negative
Flavonoids	Wilstater test	Positive

The presence of tannins, alkaloids, saponins, flavonoids, steroids, and terpenoids may be responsible for antidiarrheal activity, especially those caused by bacteria. Flavonoid compounds are phenolic groups which are known to have antimicrobial activity by inhibiting the DNA girase enzyme (Cushnie and Lamb, 2005). Meanwhile, tannins compounds have antimicrobial activity by binding to bacterial cell walls, preventing growth and inhibiting the activity of protease enzymes (Jones et al., 1994). Saponins are known to interact with cell membranes, increase permeability and produce cell damage (Francis et al., 2002). In addition, the mechanism of action of some alkaloids is associated with their ability to interact with DNA (Phillipson and O'Neil, 1989).

Table 2 : In vitro antidiarrheal activity of *R. rosifolius* leaves extract

Concentration	*E .coli*	*S. aureus*
160 mg/mL	9,333 ± 0,764^{a}	4,100 ± 0,100^{a}
320 mg/mL	10,700 ± 1,058^{a}	6,000 ± 0,500^{b}
640 mg/mL	11,333 ± 1,258^{a}	8,400 ± 0,264^{c}
1280 mg/mL	14,500 ± 1,323^{b}	9,200 ± 0,264^{d}
Ampicillin 20 µg/mL	25,000 ± 1,000^{c}	0 ± 0
Gentamicin 20 µg/mL	15,167 ± 2,021^{b}	0 ± 0

Value (mean ±SD) are average of each sample, determined in triplicate (n=3). Different letters (a-d) in the column above showed significantly different results ($P < 0.05$).

R. rosifolius leaves extract produced the highest inhibitory zone diameter at a concentration of 1280 mg/mL compared to other concentrations. This was because the antibacterial compounds contained in *R. rosifolius* leaves extract with a concentration of 1280 mg/mL had the greatest amount. One factor that influenced the amount of inhibition is the concentration of bacterial compounds contained in the extract. This was consistent with the research of Pelczar and Chan (2005) who reported that the higher the concentration of extract, the greater the activity and the effects.

The effect of treatment with extract concentrations of 160 mg/mL to 1280 mg/mL against *E. coli* and *S. aureus* bacteria experienced an increase in inhibitory zone. According to Davis and Stout (1971), the criteria for antibacterial power strength based on the diameter of the inhibitory zone are as follows: the diameter of the inhibitory zone <5 mm is classified as weak, the inhibitory zone 5-10 mm is classified as moderate, the inhibitory zone 11-19 mm is strong and > 20 mm included in the very strong class. Based on these criteria, the antibacterial strength of *R. rosifolius* leaves extract at a concentration of 160 mg/mL against *E. coli* and *S. aureus* bacteria was in the middle and weak; at a concentration of 320 mg/mL against *E. coli* and *S. aureus* bacteria included in the strong and medium class; at a concentration of 640 mg/mL against *E. coli* and *S. aureus* bacteria belonged to the strong and medium class; at a concentration of 1280 mg/mL against *E. coli* and *S. aureus* bacteria belonged to the strong and medium class.

Based on diameter of the inhibitory zone the activity of ethanol extract of *R. rosifolius* leaves in inhibiting the growth of *E. coli* bacteria which was a gram-negative bacterium was higher than that of *S. aureus* which was a gram-positive. The difference in cell wall structure of gram-positive and gram-negative bacteria may cause difference in the magnitude of inhibitory zones for various materials (Pelczar and Chan, 1998). In addition, *R. rosifolius* leaves contained β-caryophillene content (Patel, 2004). According to Deba (2008), the compound of β-caryophillene had maximum activity against gram-positive bacteria, but it had low activity against gram-negative bacteria. The mechanism of inhibition by β-caryophillene was not fully understood, but it was thought to affect lipophilic compounds in the cell membrane of bacteria (Deba et al., 2008). The difference in results was possible because of differences in the content of other phytochemical compounds.

CONCLUSION

From the results of this research, it could be concluded that the ethanol extract of Calingan leaves (*Rubus rosifolius*) had inhibitory activity against *Staphylococcus aureus* and *Eschericia coli* bacteria where the higher the concentration of *R. rosifolius* leaves extract, the greater the inhibitory zone formed against *S. aureus* and *E. coli* bacteria growth.

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Antioxidant Activity From Awar-Awar (*Ficus septica* Burm. F) Leavesethanol Extract And Pearl Grass (*Hedyotis corymbosa*)

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Abstract

Awar-awar (*Ficus septica* Burm. F) contains compounds that have antioxidant activity such as flavonoids. Flavonoids are believed to function as antioxidants by inhibiting Reactive Oxygen Species (ROS). While pearl grass (*Hedyotis corymbosa*) contains ursolic acid which is a triterpenoid group, which functioned as an antioxidant mechanism. The aim of this research is to measurethe antioxidant activity from the combination of awar-awar and pearl grass, ES50 value and awar-awar leaf ability to increase antioxidant activity from pearl grass.

The extraction of simplisia was done by maceration using 70% ethanol. The qualitative test was done visually using F-254 silica gel. Concentration used for the quantitative test on pearl grass was 20, 25, 30, 35, 40, 45 µg / mL and in combination used 10, 15, 20, 25, 30, 35 µg / mL. ES50 data was analyzed using DPPH method with UV-Vis spectrophotometer instrument. The data were compared with the result of the test using ANOVA with 95% confidence level. Results showed that the combination extract can increase the ES50 value. ANOVA analysis showed that there were significant differences between pearl grass and awar-awar leaf extract. The combination of two extracts resulted the more bigger chance to catch free radicals. The conclusion of this research were combination preparations have higher antioxidant activity when compared with non-combination, it is indicated by ES50 value of pearl grass extract and awar-awar leaf extract combination of pearl grass extract.

Keywords : Antioxidant, awar-awar (*Ficus septica Burm F.*), DPPH, pearl grass (*Hedyotis corymbosa*)

INTRODUCTION

Free radicals are one of the causes of cancer and causes of degenerative diseases. According Soetmaji (1998), which refers to free radicals are molecules or more unpaired electrons in the orbital exterior which can be customized with the components, as well as the molecules making up the membrane or functional components, include proteins, enzymes, and DNA (Hidayat, 2005). Cancer and degenerative diseases can be relieved of bodies that have free radicals (Hernani and Rahardjo, 2005). The role of antioxidants is essential to neutralize and liberate free radicals which can cause damage and damage biomolecules.

Screening of active compounds carried out on awar-awar leaves shows that there are alkaloids, flavonoids and phenolic, such as resveratrol and genistein. Flavonoid and phenolic compounds are widely known as natural antioxidants. The mechanism of flavonoid antioxidants is to capture the ROS directly, prevents the regeneration of ROS and indirectly increases the antioxidant activity of cellular antioxidant enzymes (Akhlaghi and Bandy, 2009). Flavonoids are compounds that are most effective as a scavenger of reactive species, for example, superdioksida, peroxyl radicals, and peroxynitrite by transferring the atom H + (Middleton et al., 2000; Akhlaghi and Bandy, 2009).

Other plants that had proven had an antioxidant function are pearl grass. Pearl grass is a wild plant that is often found in home gardens. Asyhar et al., (2008) have discovered Ursolic acid and acid oleanolate compounds in pearls (*Hedyotis corymbosa*). Ursolic acid included in the class pentacyclic triterpenoid compounds that can be found in most herbaceous plants and fruit trees. Sopandi *et al* (2007) statede that pearl grass has antioxidant activity in fractions of ethanol with 50% inhibitory concentration (IC50) DPPH of 56.57 mg / mL. According to Topcua et al, (2007) the method of antioxidants from triterpenoids was by capturing/searching for reactive species, such as superoxide, and chelating metals (Fe2 + and Cu2 +). Abrosca et al (2006) showed that triterpenoid compounds have activity as antioxidants and can inhibit lipid peroxide.

The method chosen in this study was the method of catching free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), because this method is a method that requires less sample, simple, easy, quick and sensitive to evaluate the antioxidant activity of the compounds of natural materials (Hanani et al., 2005).

MATERIALS AND METHOD

1. Plant Extraction

A total of 500 grams of Awar-Awar leaf powder were weighed, macerated with 2.25 liters of 70% ethanol. 400 grams Pearl grass powder macerated with 1.5 liters of 70% ethanol and left at room temperature for 5 days protected from light, while stirring repeatedly, then filtered. Then the awar-awar pulp and pearl grass were regenerated with 750 mL and 500 mL of 70% ethanol at room temperature for 2 days,than filtered (Anonim, 1986). The juice then concentrated with a rotary evaporator at a temperature of 50-60 ° C until the extract is obtained. Evaporation performed using a water bath until thick extract obtained

(Padmasari et al., 2013). A viscous extract obtained is weighed and the percentage of the yield.

2. **Determination of Water Content**
Determination of water used toluene distillation method. A total of ± 5 gram sample of the dried extract incorporated into a dry flask, then 200 mL of water-saturated toluene was added to the flask than heated gently for 15 minutes. After the toluene begins to boil, distillation speed is set ± 2 drops per second, then the distillation speed is raised to 4 drops per second. After separating the water and toluene was perfect, than the water volume can be read (Anonymous, 2008).

3. **Qualitative Test (Visual)**
Tests were carried out using silica gel F254 plate as a medium. Then a concentration of 1% was made for each sample and sprayed with 0.15 mM DPPH.

4. **Antioxidant Activity Test with DPPH**
Three test samples, namely, quercetin as standard, pearl grass extract and leaf extract as a comparison of awar awar leaf extract combination as the test sample. All samples were prepared in a concentration of 1% as the concentration of the parent. Then each sample was made a series of concentration. Concentration of quercetin is made at 2; 2.5; 3, 3,5; 4; 4.5 µg / mL. Pearl grass extracts were made at concentrations of 20, 25, 30, 35, 40, 45 µg / mL and in combination extracts were made at concentrations of 10, 15, 20, 25, 30, 35 µg / mL. The antioxidant activity by lowering the DPPH absorbance after adding the test sample. Then the ES50 price was calculated using linear regression to get 50% radical capture concentration (ES50). To calculate PRB we used equation:

$$\%\ PRB = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100\%$$

RESULT AND DISCUSSION

Extraction

Extraction results obtained from condensed extract of each plant with the amount of yield in Table I.

Table I. The yield of Awar-awar Leaf Extract and Pearl Grass Extract

Result	Awar-awar Leaf Extract	Pearl Grass Extract
Initial Weight (gr)	500	400
Extract Weight (gr)	51,60	36,548
Yield (% b/b)	10,32	9,14

Determination of Water Content

Determination of water content aims to determine the water content in the sample-awar awar leaves (*Ficus septica* Burm. F) and pearl grass (*Hedyotis corymbosa*) (Table II). Puspita and

Utami (2009) states that if the water content contained in the sample is less than 10%, then the stability of the optimum material can be achieved and the growth of microbes can be reduced.

Table II. Determination of Awar-awar Leaf Extract Water Content

Explanation	Water Content of Leaf Awar-awar (%b/v)	Water Content of Pearl Grass (%b/v)
Replication 1	6,00	7,84
Replication 2	5,88	7,98
Replication 3	5,80	7,93
Average ± LE	5,89 ± 0,892	7,93± 0,041
SD	0,101	0,071
CV	1,715	0,896

Antioxidant Identification Test

Antioxidant identification test was carried out to determine the antioxidant activity in the sample visually using silica gel F254 (Gandjar and Rohman, 2007). The results obtained are yellow spots were produced from combination extract more concentrated than the pearls grass extracts without the combination (Figure 1).

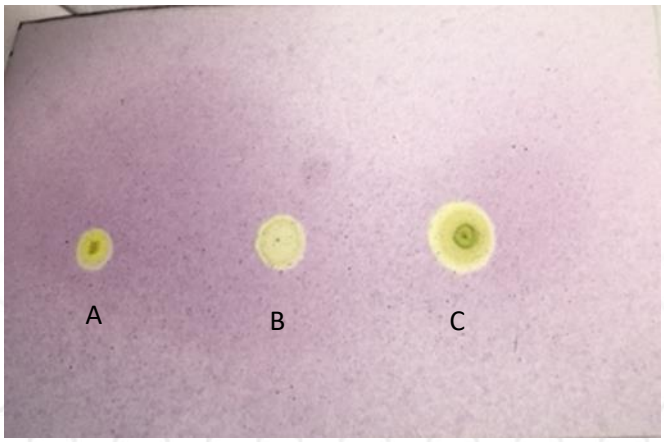

Figure 1. Spotting Antioxidants (A = Quercetin, B = Grass Pearls, C = Combination)

Yellow spots indicate the presence of antioxidant activity in the samples. In this test 1% concentration was used for each test sample and 0.15 mM DPPH as a radical source. Point A is quercetin as a standard antioxidant, point B is a pearl grass extract and point C is combination extract. The color produced by combination extract approached the yellow color of quercetin as an antioxidant standard.

Antioxidant Activity Test

Determination of the maximum absorption wavelength aims to determine the wavelength that has maximum absorbance. This study uses quercetin standard solution with a concentration of 10 mg / mL to measure the wavelength of maximum absorption and the wavelength of maximum absorption is obtained which is 433.0 nm. Where the theory of maximum wavelength of quercetin is 315-515 nm (Selawa et al., 2013).

Antioxidant Activity Test Results

In quantitative terms, the ability of the sample in a muffle shown with decreasing DPPH radical absorbance test solution calculated against the reference solution (Table III). The smaller the absorbance value, the stronger the ability of the test sample to reduce DPPH (Sanchez, 2002). The data obtained from the measurement is Effective Scavenging (%) and the concentration of the test compound then processed using a linear regression analysis to obtain 50% effective radical capture (ES50). Suryanto et al, (2003) use the term ES50 for the interpretation of the results of DPPH.

Table III. Antioxidant Activity Test Results of Quercetin with DPPH Method

Concentration	% Percent of Free Radical Arrest (DRR)				
	R1	R2	R3	R4	R5
2	100,840	117,647	138,655	96,639	106,443
2,5	162,465	179,272	182,073	198,880	215,686
3	250,700	263,305	252,101	260,504	260,504
3,5	324,930	329,132	308,123	313,725	319,328
4	362,745	365,546	368,347	366,947	368,347
4,5	422,969	425,770	413,165	424,370	432,773
Equation Regression	y = 13,061x - 15,372 R=0,994	y = 12,373x - 12,201 R=0,994	y = 11,357x -9,201 R=0,998	y = 12,549x - 13,100 R=0,992	y = 12,277x - 11,515 R=0,990
ES_{50} (µg/mL)	**5,01**	**5,03**	**5,21**	**5,03**	**5,01**
Average	5,058 ± 0,7195 µg/mL				
SD	0,0856				
CV	1,692				

Antioxidant specifications are divided into four, namely if the antioxidant is very strong has an ES50 value <50 µg / mL, strong antioxidants have ES50 values between 50-100 µg / mL, moderate antioxidants have ES50 101-250 µg / mL, weak antioxidants have ES50 251 -500 µg / mL and inactive antioxidants have ES50 values> 500 µg / mL (Ahmad et al., 2013). In other words, the higher the value, the smaller ES50 a compound's ability to capture free radicals DPPH.

Table IV. Antioxidant Activity Test Results of Pearl Grass Ethanol Extract with DPPH Method

Concentration	% Free Radical Arrest (DRR)				
	R1	R2	R3	R4	R5
20	14,9194	11,6935	13,7097	11,0215	11,8280
25	17,4731	20,6989	21,6398	22,4462	21,5054
30	25,5376	29,7043	29,8387	28,6290	26,4785
35	31,7204	32,6613	33,7366	34,2742	33,8710
40	38,9785	38,1720	39,9194	39,1129	41,9355
45	47,7151	52,5538	49,7312	47,8495	50,6720
Regression Equations	y=1,341x -14,192 R=0,992	y=1,484x -17,312 R=0,982	y=1,370x -12,928 R=0,994	y=1,370x -13,976 R=0,990	y=1,502x - 17,776 R=0,997
ES_{50} (µg/mL)	**47,87**	**45,36**	**46,10**	**46,70**	**45,12**
Average ± LE	46,23 ± 0,302 µg/mL				
SD	1,109				
CV	2,399				

Table V. Antioxidant Activity Test Result of Ethanol Extract of Leaf Awar-awar Combination of Ethanol Extract of Pearl Grass (1: 1) with DPPH Method

Concentration	% Free Radical Arrest (DRR)				
	R1	R2	R3	R4	R5
10	85,020	86,370	67,476	68,830	67,476
15	209,177	207,827	206,478	207,827	218,623
20	295,547	299,595	299,595	300,945	299,595
25	373,819	380,567	455,655	387,314	379,217
30	454,791	457,490	465,587	488,529	481,781
35	510,121	515,520	529,015	531,714	522,267
	y = 1,680x -5,667 R=0,993	y = 1,700x - 5,803 R=0,993	y = 1,852x - 7,941 R=0,973	y = 1,853x - 8,608 R=0,991	y = 1,796x -7,595 R=0,988
ES_{50} (µg/mL)	**33,14**	**32,83**	**31,29**	**31,63**	**32,07**

Average	32,192 ± 0,435 µg/mL
SD	0,783
CV	2,432

The following are ES50 values in quercetin standard, ethanol extract of pearl grass, and ethanol extract of awar-awar leaves a combination of pearl grass ethanol extract (1: 1) respectively 5.058 ± 0.7195 µg / mL, 46.23 ± 0.302 µg / mL; and 32,192 ± 0,435 µg / mL (Figure 2).

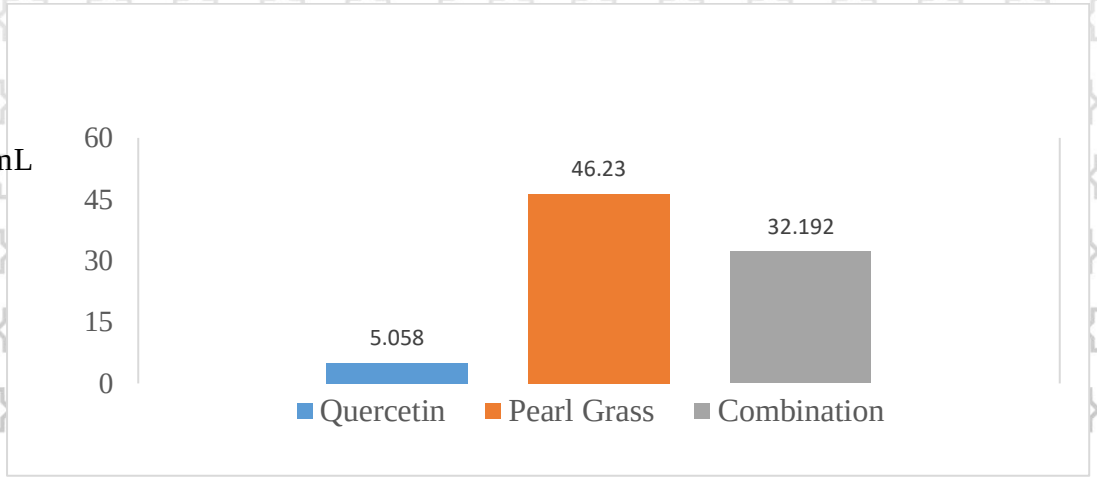

Figure 2. Comparison of ES50 value of Quercetin, Ethanol Extract of Pearl Grass, and Ethanol Extract of Awar-awar Leaf Combination of Pearl Grass (1: 1)

The equation specification test results

The next test was ANOVA test at 95% confidence level. The ANOVA method was used to compare more than 2 test groups, so it is known whether between groups have significant differences or not (Wiratna, 2015). In the test of significance value of 0.000 where the significance value less than 0.05 so it can be concluded that Ho is rejected or between treatment groups were significantly different. The difference in ES50 values above proved to be significantly different in all treatment groups ($p < 0.05$) with ANOVA test. Testing the antioxidant activity of ethanol extracts of pearl grass and combination extract showed that the combination extract had better free radical scavenging activity. This is because the presence of some active compounds contained in extracts of leaf-awar awar that can synergize in inhibiting free radicals, so the value of the ES50 in combination extract beter than a single extract (without combination). Although in ethanol extracts of pearl grass and extracts of different combinations significantly by SPSS analysis, in general the antioxidant activity of both groups was included in the very strong category (<50 µg / mL) (Ahmad et al., 2013).

CONCLUSION

Ethanol extract of awar-awar leaves can increase antioxidant activity of pearl grass ethanol extract, as evidenced by ES50 value of the combination extract (1: 1) of 32.192 µg / mL while the ethanol extract of pearl grass without combination was 46.23 µg / mL.

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Hydroxyl Radical Scavenging Activity and Colagenase Inhibitory Activity of *Curcuma mangga* Ekstrak and its Fractions

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Abstract

Curcuma mangga has bioactive compound such as curcumin. Curcumin is a natural antioxidant and known as antiaging agent. Curcumin can stimulate the synthesis of collagen type I, inhibit melanogenesis, and help to maintain correct skin hydration. This show *C. mangga* has potential as antioxidant and antiaging agents. This research aims to find out the antioxidant and antiaging activities of *C. mangga* ethanolic extracts (CMEE) and its fractions. In this study, the antioxidant activity of CMEE and its four fractions (water, hexane, ethyl acetate, and butanol fraction) was measured by hydroxyl radical scavenging assay, while the antiaging activity were performed by measuring inhibition of collagenase as inhibitory activity assay. Ascorbic acid using as a control. The hydroxyl radical scavenging activity show ascorbic acid has the highest activity (28.04 µg/mL) but CMEE also has high value (40.24 µg/mL) compared to other fractions. CMEE has the highest collagenase inhibitory activity (19.65 µg/mL) compared to fractions and ascorbic acid (94.69µg/mL). In summary, *C. mangga* extracts (CMEE) has antioxidant activity due to hydroxyl radical scavenging activity**, whit the mechanisme as** anticollagenase and has good antiaging.

Keywords: *C. mangga*, Antiaging, Anticollagenase.

INTRODUCTION

Aging of the skin is induced by both intrinsic, and extrinsic factors (Farage, et al., 2008). Reactive oxygen species (ROS) play an important role in skin aging. In the skin, about 1.5-5% of the consumed oxygen is converted into ROS by intrinsic processes (Poljsak, et al., 2012). Two other main events associated with intrinsic skin aging are a decrease in replicative ability of cells and increased degradation of the extracellular matrix (Rinnerthaler, et al., 2015). Aging has a quite different appearance depending if either dermis or epidermis is considered. In the dermis the disruption of the extracellular matrix plays the most obvious role which is true for intrinsic as well as extrinsic aging. The results are fine wrinkles due to the reduction of collagen, elastic fibers, and hyaluronic acid (Rinnerthaler, et al., 2015). Elastase, collagenase, hyaluronidase and tyrosinase, are very interesting enzymes due to their direct implication in skin aging and as therapeutic hits (Fayad, et al., 2017).

Collagenase is an enzyme that plays an important role in degradation of collagen. Collagen is the main component with percentage of 70%-80% of the total skin weight. The increasing degradation of collagen is significant in aging. Hyaluronidase inhibitors are therefore effective regulating agents, that maintain the balance between the anabolism and catabolism of HA and this keep skin moist and well as smooth (Liyanaarachchi, *et al*., 2018). Tyrosinase (tyr), the key protein involved in skin pigmentation due to the presence of melanin pigment, is activated and a hyperpigmentation is produced (Videira, *et al*., 2013). Aging also can be prevented by scavenging free radicals such as hydroxyl radical (OH). The hydroxyl radical (OH) is one of the most powerful oxidizing agents, able to react unselectively with the surrounding chemicals, including organic pollutans and inhibitors (Gligorovski, et al., 2015).

Curcuma mangga is commonly called “temu mangga” or “kunir putih” in Indonesia belongs to Zingiberaceae family (Indis & Kurniawan, 2016). The genus *Curcuma* is widely distributed in tropical Asia and Australia (Baharudin, et al., 2015). *C. mangga* rhizomes is the one kind of herbal medicinal plants contain some bioactive compounds such as curcuminoid (curcumin, bisdemethoxycurcumin, bismethoxycurcumin) and phenolic (Indis & Kurniawan, 2016). The rhizome of *C. mangga* has been reported to possess some pharmacological activities such as antioxidant (Indis & Kurniawan, 2016) and anticancer (Hong, et al., 2016). Thus, in this study we used *C. mangga* ethanol extracts and its fractions to evaluate the antioxidant and antiaging activities through hydroxyl scavenging activity and inhibitory of collagenase, hyaluronidase, elastase and tyrosinase activity.

MATERIALS AND METHODS

Preparation of C. mangga Extract

C. mangga plants were collected from the plantation located in Yogyakarta, Special Region of Yogyakarta, Indonesia. The extraction was performed by maceration method. *C. mangga* was dried, blanched, and milled then soaked in 70% distillated ethanol. The substance was filtered every 24 hours until colorless filtrate was gained. After that, the filtrate was evaporated using rotary evaporators (Stuart, RE 300) to obtain ethanol extract, after it was done, the *C.*

mangga extract (CME) was stored at -20 °C (Widowati, et al., 2016; Widowati, et al., 2017; Widowati, et al., 2018).

Fractionation of C. mangga Ethanol Extract

Fractionation of *C. mangga* ethanol extract was done using modified partition. *C. mangga* ethanol extract (25 g) was partitioned with n-hexane and water (1:1), obtained a hexane fraction of 3.77 g (18.85%), then the residue was partitioned with ethyl acetate and water (1:1), obtained an ethyl acetate fraction of 4.62 g (9.24%); the residue was then partitioned with butanol and water (1:1), obtained a butanol fraction of 2.40 g (4.8%); and the remaining residue was the water fraction of 11.93 g (23.86%) (Widowati, et al., 2011) (Soeng, et al., 2015) (Tjahjani, et al., 2014) (Pujimulyani, et al., 2018).

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the extracts was determined according to the method reported by Halliwell *et al*. (1987). The reaction mixture contained 30 µL of different concentration of sample, 10 µL $FeCl_3$-EDTA, 5 µL H_2O_2 20 mM, 5 µL L-ascorbic acid 1 mM, 10 µL Deoxyribose (2-Deoxy-D-ribose) 2.8 mM, and 70 µL buffer. There was incubated in 37 °C for 30 min. Then added 25 µL TCA 5%, TBA 1%, and then incubated 80-90 °C for 30 min. Absorbance was measured at 532 nm wavelength (Halliwell, *et al*., 1987).

Collagenase inhibitory activity assay

Inhibition of collagenase activity was measured based on the method that was elaborated by Sigma Aldrich and Thring et al. (2009) with some modifications. The assay was performed by dissolving 10 µL collagenase enzyme from *Clostridium histolyticum* (Sigma C8051, USA) (0.01 U/ mL in cold distilled water), 60 µL buffer Tricine (50 mM, pH 7.5, contains 10 mM $CaCl_2$ and 400 mM NaCl), 30 µL sample (0-250 µg/mL in DMSO). The mixtures were incubated for 20 min at 37˚C. After incubation time, 20 µL substrate N-[3-(2-Furyl)acryloyl]-leu-gly-Pro-Ala (FALGA) (Sigma F5135, USA) (1 mM in buffer *Tricine*) was added. Absorbance at 335 nm wavelenght was measured immediately after adding the substrate (Thring, et al., 2009; Widowati, et al., 2017; Widowati, et al., 2016; Utami, et al., 2018).

% Collagenase inhibition = (1-B/A) x 100%

A = Sample absorbance

B = Control absorbance

Statistical analysis

In this study, the statistical analysis was performed using SPPS software (version 17.0). The data were analyzed by analysis of variance (ANOVA) continued with Tukey HSD post hoc test, $p<0.05$ was considered as statistically significant. Values are expressed as means ± standard deviation.

RESULTS

In this study, the antioxidant and antiaging properties of CMEE and those fractions (from 4 solvents) were assessed as hydroxyl radical scavenger while antiaging activities due to collagenase, elastase, hyaluronidase, and tyrosinase inhibitory activity.

Hydroxyl radical scavenging activity

Antioxidant activity of CMEE and its fractions were assessed using the hydroxyl radical scavenging activity. Hydroxyl radical scavenging activity of CMEE and its fractions was performed in Fig. 1 and Table 1.

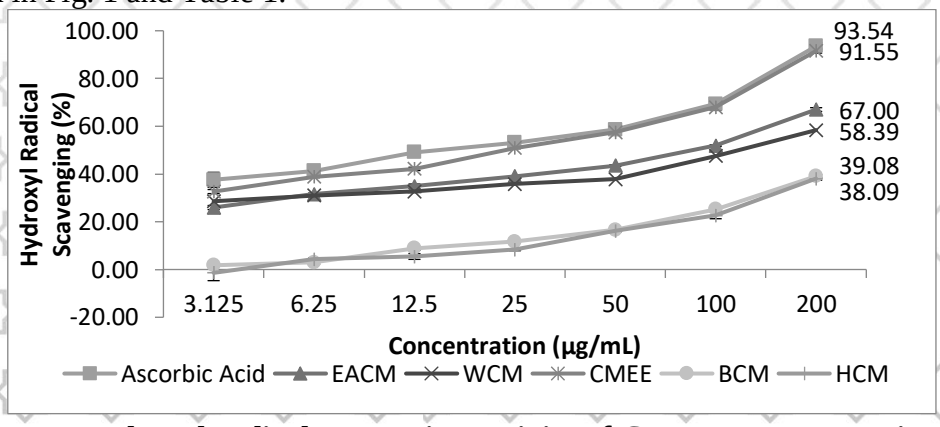

Figure 1. Hydroxyl radical scavenging activity of *C. mangga* extracts, its fractions and ascorbic acid.

*CMEE= *C. mangga* ethanol extracts, WCM= Fraction of *C. mangga* water, EACM= fraction of *C. mangga* ethyl acetate, BCM= fraction of *C. mangga* butanol , HCM= fraction of *C. mangga* hexane.

In Fig. 1, the hydroxyl radical scavenging activity of some *C. mangga* fractions was determined with compared to ascorbic acid. However, CMEE completely can inhibit hydroxyl radical with presentation 91.55% . The extract of *C. mangga* (CMEE) has the highest compared to other fractions of *C. mangga*, but not higher than ascorbic acid (93.54%) in the highest concentration (200 µg/mL) ($p<0.05$) (Fig. 1).

Table 1. IC_{50} value of Hydroxyl radical scavenging activities by *C. mangga* extracts, its fractions and ascorbic acid

Samples	Equation	R^2	Average IC_{50} (µg/mL)
Ascorbic Acid	y = 0.2616x + 42.666	0.96	28.04
CMEE	y = 0.2754x + 38.918	0.95	40.24
WCM	y = 0.1458x + 30.569	0.98	133.27
EACM	y = 0.188x + 31.319	0.95	99.37
BCM	y = 0.1807x + 4.9225	0.96	303.94
HCM	y = 0.1843x + 2.9132	0.96	287.10

*CMEE= *C. mangga* ethanol extracts, WCM= Fraction of *C. mangga* water, EACM= fraction of *C. mangga* ethyl acetate, BCM= fraction of *C. mangga* butanol , HCM= fraction of *C. mangga* hexane; IC50= The half maximal inhibitory concentration. IC50 of the samples were calculated.

Table 1 show that CMEE has the lowest IC_{50} value (40.24 µg/mL) compared to *C. mangga* fractions but not higher than ascorbic acid (28.04 µg/mL). This indicated CMEE has good antioxidant activity through hydroxyl radical scavenging activity.

C. mangga or white saffron is medicinal herb that has many bioactive compounds. In the present study, the fraction of *C. mangga* extracts and those fractions were evaluated in antioxidant and antiaging activity assay. *C. mangga* has been known has potential in antioxidant (Nahak & Sahu, 2011) and antiaging agent (Mukherjee, et al., 2011). Ethanol extracts of *C. mangga* and its fractions (aqueous, chloroform, ethyl acetate, and hexane) has anti-inflammatory and the inflammation conditions it effect for skin appearance (Ruangsang, et al., 2010) (Kusumawati, et al., 2018).

As shown in this result study, CMEE has the highest in hydroxyl radical scavenging activity (IC_{50}=40.24 µg/mL) compared to all *C. mangga* fractions. The fractions of *C. mangga* ethanol extract has potential as antioxidant agent through NO and H_2O_2 scavenging activities (Pujimulyani, et al., 2018). *C. mangga* has curcuminoids compound, such as curcumin (Yuandani & Yuliasmi, 2018). Curcumin is capable of scavenging oxygen free radicals such as superoxide anions and hydroxyl radicals, which are the initiators of lipid peroxidation. Curcumin is reported to be a powerful antioxidant to repair both oxidative and reductive damage caused to proteins by radiation (Kapoor & Priyadarsini, 2001). Curcumin has mechanism of antioxidant, in the presence of ethyl linoleate as one of the polyunsaturated lipids was reported (Masuda, et al., 2001). The scavenging of the hydroxyl radicals may be due to the presence of hydrogen donating ability phenolic compounds in the extracts (Pavithra & Sasikumar, 2015)

Collagenase Inbitory Activity

Anti-collagenase activities were observed for CMEE and its fractions and the result were performed in Fig. 2 and Table 2.

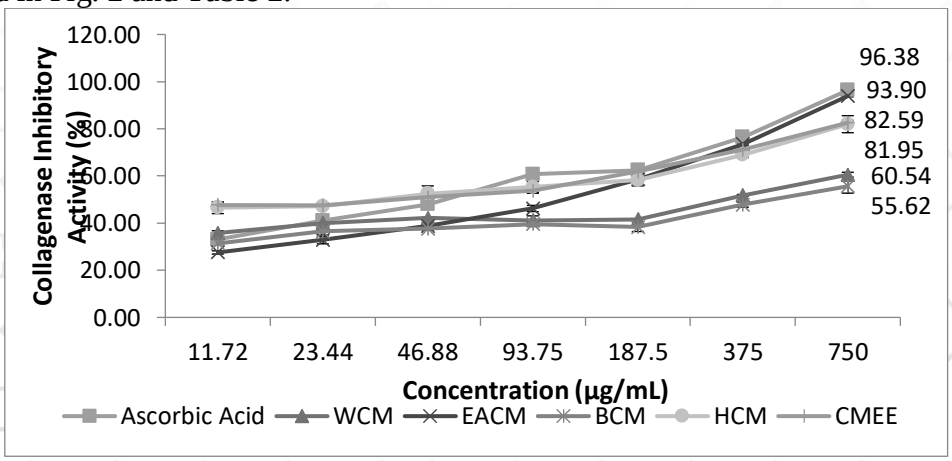

Figure 2. Collagenase inhibitory activity of *C. mangga* extracts, its fractions and ascorbic acid.

*CMEE= *C. mangga* ethanol extracts, WCM= Fraction of *C. mangga* water, EACM= fraction of *C. mangga* ethyl acetate, BCM= fraction of *C. mangga* butanol , HCM= fraction of *C. mangga* hexane.

As shown in Fig. 2, EACM has the highest in collagenase inhibitory activity (93.90%) compared to other fractions and CMEE, but not higher than ascorbic acid (96.38%) in concentration 750 µg/mL. In addition, good activities as antaging were exhibited by EACM compared to other fraction and CMEE.

Table 2. IC_{50} value of collagenase inhibitory activities by *C. mangga* extracts, its fractions and ascorbic acid

Samples	Equation	R^2	Average IC_{50} (µg/mL)
Ascorbic Acid	y = 0.0776x + 42.485	0.92	96.84
CMEE	y = 0.0484x + 49.049	0.96	19.65
WCM	y = 0.0306x + 38.233	0.94	384.54
EACM	y = 0.086x + 34.752	0.93	189.18
BCM	y = 0.029x + 34.811	0.92	500.31
HCM	y = 0.0467x + 48.744	0.97	26.90

*CMEE= *C. mangga* ethanol extracts, WCM= Fraction of *C. mangga* water, EACM= fraction of *C. mangga* ethyl acetate, BCM= fraction of *C. mangga* butanol , HCM= fraction of *C. mangga* hexane; IC50= The half maximal inhibitory concentration. Linear equations, coefficient of regression (r2) and IC50 of the samples were calculated.

Based on Table 2, CMEE has the highest collagenase inhibitory activity (19.65 µg/mL) compared to those of fractions and ascorbic acid (96.84 µg/mL) ($p<0.05$). This would mean that CMEE had significantly highest in collagenase inhibitory activity compared to those fractions.

In this study, we determine about collagenase inhibitory activity of *C. mangga* extracts (CMEE) and its fractions. CMEE has the lowest IC_{50} value (19.65 µg/mL) compared to ascorbic acid (94.69µg/mL) and other *C. mangga* fractions. The curcuminoid content in CMEE may correlated in collagenase inhibitory activity. Curcuminoid content in Curcuma genus was correlated with antiaging activity, the *C. heyneana* crude extract produced significance effects on the UV-induced skin structure damage (Kusumawati, et al., 2018). Thus, curcumin in *Curcuma* extracts has collagenase inhibitory activity (Kusumawati, et al., 2018). Curcumin also can induces cellular stress responses in normal human skin fibroblasts through phosphatidylinositol 3-kinase/Akt pathway and redox signaling, the stimulation hormetic of curcumin from cellular antioxidant defenses can be a useful approach toward antiaging intervention (Lima, et al., 2011). Besides curcumin, xanthorrhizol (terpenoid compound) that isolated from *C. xanthorrhiza* was involved in the expression of MMP-1 and type-I procollagen in UV-irradiated human skin fibroblasts (Oh, et al., 2009). Xanthorrhizol and *C. xanthorrhiza* extract (0.01–0.5 g/ml) induced a significant, dose-dependent decrease in the expression of MMP-1 protein and increased the expression of type-1 procollagen (Mukherjee, et al., 2011).

CONCLUSION

In summary, *C. mangga* extracts (CMEE) has antioxidant activity due to hydroxyl radical scavenging activity**, whit the mechanisme as** anticollagenase and has good antiaging**.** Thus present data suggest that *C. mangga* ethanolic extract and its fractions can be used as a good source of natural antioxidant and antiaging for health benefits. In future study, the further isolation of bioactive compounds from *C. mangga* are required for identifying the unknown compounds to establish their pharmacological properties.

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Conflict of interest

The authors declare that they have no competing interests.

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ANALYSIS OF THE DETERMINANT FACTORS OF CERVICAL CELL RISK IN PRE-ELDERLY AND THE ELDERLY WOMEN

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Abstract

Background: Indonesian women at the community level experience two serious conditions when they reach pre-elderly and elderly, namely cardiovascular disease and cervical cancer. Theoretically the link between these two conditions is chronic inflammation. Until now there has been no research that gives particular attention to the relationship between this two clinical conditions.
Objective: This study aimed to analyze whether determinant factors determine the risk of morphology of cervical cells in women undergoing Pap smear examinations.
Method: This study used a cross sectional design, analyzing determinant factors which included climacterial status, stress level, cardiovascular risk, body mass index (BMI), contraception type, number of parity, age at marriage, and level of education. Determination of morphological classification of cervical cells using the Bethesda assessment standard. Statistical analysis using chi square.
Results: Two determinant factors were obtained that were statistically significant, namely cardiovascular risk (OR = 1,839; p = 0.030 and stress level (OR = 0.501; p = 0.017).
Conclusions: The increase in cardiovascular scores correlates with the level of systemic inflammation correlated with the worsening morphological quality of cervical cells, increased stress levels but under clinical conditions are protective factors.

Keywords: Cardiovascular risk, morphological cervical cell, Pap smear

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INTRODUCTION

In the world today it is estimated that every year there are 18.1 million new cancer cases and 9.6 million cases of cancer that have died. Of these cervical cancers ranked 10th, which amounted to 569,847 people (3.2%) with a mortality rate of 311,365 (3.3%). Cervical cancer is a deadly group of cancers (Bray et al, 2018). In general the prevalence of female cancer in Indonesia is higher than men 2.85 compared to 0.74 (Ministry of Health of the Republic of Indonesia, 2019). The number of cervical cancer patients in Sukoharjo district in 2015 was 41 people, while in 2016 there were 21 people (Sukoharjo District Health Office 2016; 2017).

The general principle in handling cancer, including cervical cancer or cervical cancer is the earlier detected, the more localized cancer colonies means it has not metastasized, the greater the chance to be treated better (Friedlander and Grogan, 2002; Jordan et al, 2009). Experience in America published in 2010, 55 million pap smear examinations per year have been conducted, ± 3.5 million abnormal results (6%), 11,070 new cases of cervical cancer, and 3870 deaths secondary to cervical cancer (Gamble et al. , 2010). Nationally, the coverage of early detection of cervical cancer by the IVA method is still very low at 2.978%. Central Java Province itself is still at 1.514% (Ministry of Health of the Republic of Indonesia, 2018). Reflecting on experience in the United States, the main concern in controlling cervical cancer is that in terms of screening, especially those who use the cytopathology method with pap smears are still very limited.

The prevalence of heart disease and its risk in women in Indonesia is 1.6% higher than 1.3%, diabetes prevalence 1.78 vs 1.21, hypertension prevalence, total cholesterol disturbed 33.9% vs 23.7%, hypertension 10.95 vs 5.74 (Ministry of Health of the Republic of Indonesia, 2019)

From the processed data from basic health research (Ministry of Health of the Republic of Indonesia, 2019), there was a significant correlation between the prevalence of cancer and the prevalence of heart disease (r = 0.558 p = 0.01), cancer with diabetes also showed the same level of correlation (r = 0.558, p = 0.01), as well as cancer with hypertension (r = 0.471, p = 0.05). Mechanically biological evidence is found that chronic inflammation is a shared mechanism that might explain the epidemiologic correlation (Haroon et al, 2012). Chronic inflammation in cancer generates two pathways to the adaptive immune system. That is mediated by Th1 lymphocytes (cellular immunity) and Th2 lymphocytes (humoral immunity) (Carvalho et al, 2014). Studies comparing healthy individuals with cancer have a Th1 deficit, whereas the secretion of Th2 cytokines has increased production (Krohn et al, 2011). Chronic inflammation can cause the production of ROS (reactive oxygen species) and metabolites such as malondialdehyde in the affected cells which can induce DNA damage and mutations, as the end result is carcinogenic (O'Byrne & Dalgleish, 2001). The further mechanism of promotion towards cancer is through the activation of transcription factors namely NFkB, STAT3, and AP1 (Activator Protein 1). With the Hallmark of Cancer approach from Hannahan, inflammatory cells from natural immunity play a role in promoting cancer growth, while adaptive immunity has the effect of suppression (Aivaliotis et al, 2012). Chronic systemic inflammation especially proinflammatory cytokines are secondary triggers of atherosclerosis, while the primary triggers of atherosclerosis are various forms of oxidized fat mainly oxidized LDL [oxLDL] (Tedgui and Mallat, 2006). Other factors that contribute to the cause of chronic systemic inflammation are stress levels, and obesity (Haroon et al, 2012).

Research that analyzes risk factors between cervical cytology changes as a marker of the onset of cervical cancer and the shared pathophysiological mechanism of chronic inflammation is still limited. This study aims to examine the determinant factors of the risky cervical cytology pattern.

METHOD

The design of this study was cross sectional involving the subjects of the study from the elderly posyandu in the village of Gumpang and Makamhaji which was the working area of the Kartasura Health Center, Sukoharjo Regency, Central Java Province. The factors analyzed in this study were climacterium status (menopause vs. not menopause), stress level (measured by DASS 42), cardiovascular risk (measured using Jakarta cardiovascular score), body mass index (BMI), type of contraception (hormonal vs non-hormonal), number of parity (2 children / less vs. more), age at marriage (20 years / less vs. 20 years), and education level (9 years vs. basic education). Determination of the morphological classification of cervical cells is risky or not, using Bethesda assessment standards (Nayar and Wilbur, 2015). Statistical analysis using chi square.

RESULT AND DISCUSSION

Univariate data of respondents is presented in table 1. Bivariate analysis is presented in table 2.

Table 1. Univariate data of respondents

Variable	Frecuency (%) / rerata (SD; min – max); N=69
Age	49.46 (9.36; 29 – 71)
Education level	
• ≤ 9 years	16 (23.2 %)
• > 9 years	53 (76.8 %)
Type of Contraception	
• Hormonal	46 (66.7 %)
• Non Hormonal	23 (33.3 %)
Menopause condition	
• Menopause	29 (42 %)
• Not yet menopause	40 (58 %)
Menarche	13.62 (1.46; 10 – 17)
Marital Age	22.71 (4.93; 12 – 47)
Number of Parity	
• ≤ 2	27 (39.1 %)
• > 2	42 (60.9 %)
Body Mass Index	25.93 (4.45; 18 – 37)

• ≥ Overweight	39 (56.5 %)
• ≤ Normal	30 (43.5 %)
DASS42	14.07 (1.087; 0 – 54)
• On Average	31 (44.9 %)
• Below average	38 (55.1 %)
Jakarta cardiovaskular score	2.57 (3.1; -3 – 10)
• Moderate/ severe risk	40 (58 %)
• Mild risk	29 (42 %)
Cervical morphology score	1.27 (1.18; 0 – 3)
• At Risk	31 (44.9 %)
• Less/ No Risk	38 (55.1 %)

Table 2. Bivariate analysis of risk factors for cervical cell abnormalities on a Pap smear

Variabel	Rujukan	Frequency (%) of cervical cell morphological abnormalities	OR	95 % CI	*P*
Menopause	Not yet menopause	17 (58.6 %)	1.737	0.985 – 3.060	0.052
DASS	Low rerata	9 (29 %)	0.501	0.272 – 0.926	0.017*
Kardiovaskuler Risk	Low	24 (60 %)	1.839	1.201 – 2.794	0.03*
Body Mass Index	Non obese/ overweight	19 (48.7 %)	1.165	0.772 – 1.757	0.470
Kontraception	Non hormonal	19 (41 %)	0.863	0.611 – 1.219	0.392
Paritas	≤ 2 child	11 (40.7 %)	0.843	0.461 – 1.541	0.575
Marriage Age	> 20 years	3 (23.07 %)	1.226	0.982 – 1.531	0.079
Education	≥ 9 years	6 (37.5 %)	0.735	0.301 – 1.798	0.496

From the bivariate analysis, there were only two variables which showed a significant level of statistical significance, namely the stress level and cardiovascular risk.

Discussion

Two factors that influence cervical cell morphology are cardiovascular risk and stress levels. There are two different directions in the direction of the relationship. Women who have cardiovascular risk also have the potential to cause cervical cell morphology at risk with the strength of the relationship 1,839 times compared to women who are not at risk of cardiovascular disease. This result is in line with the initial hypothesis and the theoretical basis

of chronic systemic inflammation is a shared pathophysiological mechanism for two events of cardiovascular disease and cancer. While the increase in stress levels, but still under clinical conditions, is a protective factor. This phenomenon can be explained by the concept of hormesis, where a mild increase in stress levels is a protective factor. The increase in stress levels exceeds clinical conditions is a risk factor, with the shared pathophysiological mechanism of chronic systemic inflammation.

CONCLUSION

An increase in the level of cardiovascular risk correlates with an increase in the level of systemic inflammation correlated with the worsening morphological quality of cervical cells, increased stress levels but under clinical conditions is a protective factor.

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THE MOLECULAR PHENOTYPE OF HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR2 JAVA ETHNIC BASED ON BODY MASS INDEX IN BREAST CANCER

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Abstract

Background: Breast cancer is the most common malignancy in women. Breast cancer is the second leading cause of death after lung cancer. More than 30% of cancer deaths can be prevented by modifying or avoiding risk factors, one of which is obesity (BMI> 27 kg / m^2). Immunohistochemical examination (IHC) is used to determine the presence and status of HER2, ER and PR proteins in the breast. The positive HER2 subtype is found to be about 15-20% and from the results of HER2 IHC examination, it can be known the prognosis of cancer as well as the therapy that can be given. Objective: To determine the correlations between body mass index (BMI) with molecular phenotype of human epidermal growth factor receptor 2 (HER2) java ethnic based in patients with invasive breast carcinoma of no special type (NST). Method: The research was conducted by cross sectional method and using retrospective approach. Result: Based on the results of the research, it was found that the majority of samples had normal category BMI (66.4%), with the dominant molecular phenotype HER2 +3 (39.8%), ER- (70.3%), PR- (77,3%), aged> 40 years (71,9%), high school education (40,6%), house wife (58,6%) and majority married status (98,4%) . Based on statistical calculation with Gamma correlation test, p = 0,590 (p >0,05) shows that correlation between BMI and HER2 is not significant. The value of r = -0.069 indicates the direction of negative correlation which can mean that the higher of BMI will make result of the lower HER2. Conclusion: There is correlation between higher BMI with lower HER2 in Java ethnic based result with moderate correlation strength

Keywords : BMI, Breast cancer, HER2, NST.

INTRODUCTION

Breast cancer is the most common malignancy in women and is the second leading cause of death after lung cancer. There are 226,000 women suffering from invasive breast cancer in 2012 in the United States. As many as 63,000 of them are carcinoma in-situ and around 40,000 women die from the disease (Kumar et al., 2015). Data from the International Agency for Research on Cancer (IARC) shows that in 2012 the percentage of new cases of breast cancer was 43.3% and the percentage of deaths was 12.9%. Indonesia as an ASEAN country has the highest breast cancer mortality rate around 36.2 / 100,000 population per year (Ening and Widiana, 2015). Other findings suggest that more than 70% of breast cancer patients come in advanced cases. This cancer case in Central Java Province ranks second as much as 8.1% after the Special Region of Yogyakarta (9.6%). In Central Java there were 12,281 cases (50.74%), with the highest population of sufferers in the City of Surakarta (Romadhon, 2013).

Several factors related with breast cancer such gene mutations, first-degree relative with history of breast cancer, race or ethnicity, age, age of menarche, age at first birth, history of benign tumors, estrogen exposure, breast density, radiation exposure, breast cancer contralateral or endometrium, diet, obesity, lack of exercise, duration of breastfeeding, and toxins in the environment (Kumar et al., 2015).

According to WHO (2015), more than 30% of cancer deaths can be prevented by modifying or avoiding risk factors, one of which is overweight or obese (Amalia et al., 2015). Based on the Indonesian Ministry of Health's Practical Guidelines for Medical Nutrition Therapy (2003) a person can be categorized as obese if they have a BMI of more than 27 kg / m2. Measurements that can be used to assess nutritional status is by using a ratio of body weight to height. This measurement is called the Body Mass Index (Oktaviana, 2011).

Immunohistochemical examination (IHK) is used as the first step in determining the diagnosis, therapy and prognosis for breast cancer patients. Cell subtypes of breast cancer that have been identified using a biological marker profile, are processed in a complex manner and then will show the presence or absence of estrogen receptors (ER + / ER-), progesterone receptors (PR + / PR-) and Human Epidermal Growth Factor Receptor2 or commonly called HER2+ / HER2- (Ening and Widiana, 2015). The positive HER2 subtype was found around 15-20% of breast cancer subtypes (Ening and Widiana, 2015) and from the results of HER2 examination, it can be seen the prognosis of cancer as well as hormonal therapy that can be given such as giving targeted therapy (Labellapansa *et al*., 2013)

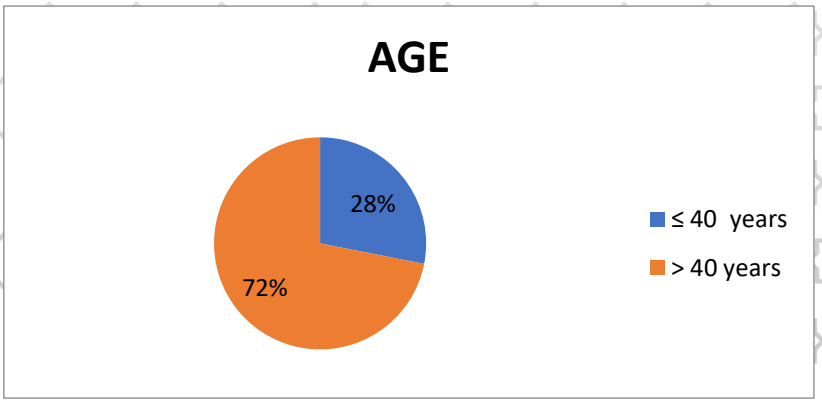
METHOD

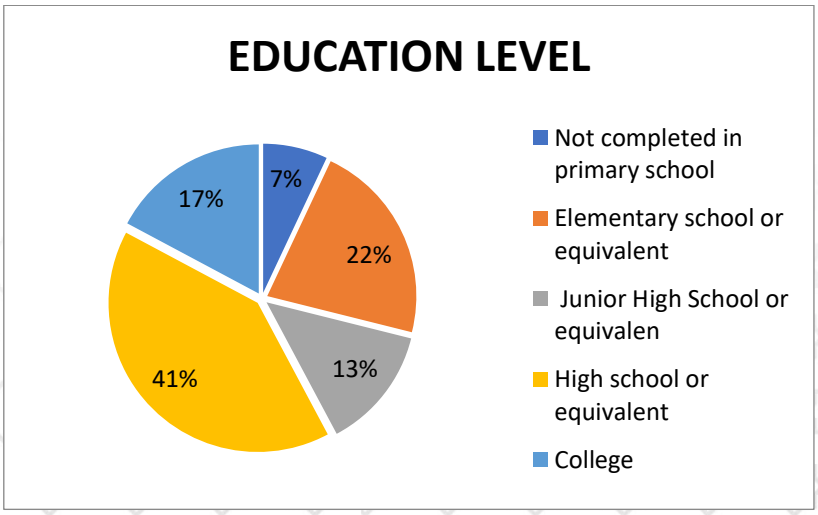
This type of research is observational analytic using a cross sectional approach. This research was conducted at the Anatomy Pathology Laboratory in the Muhammadiyah University and the medical record section from a hospital at Surakarta City. The sampling technique used in this study is Purposive Sampling. The sample came from 128 Javanese ethnic women,

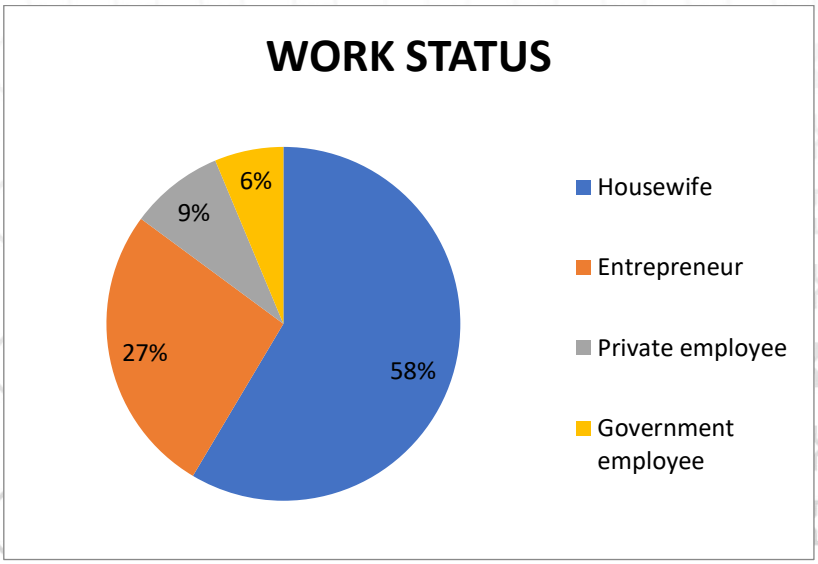
histopathology showed invasive breast carcinoma of a special type (NST) and had HER2 examination results. Data obtained from the results of the study were processed by the correlative Gamma hypothesis test.

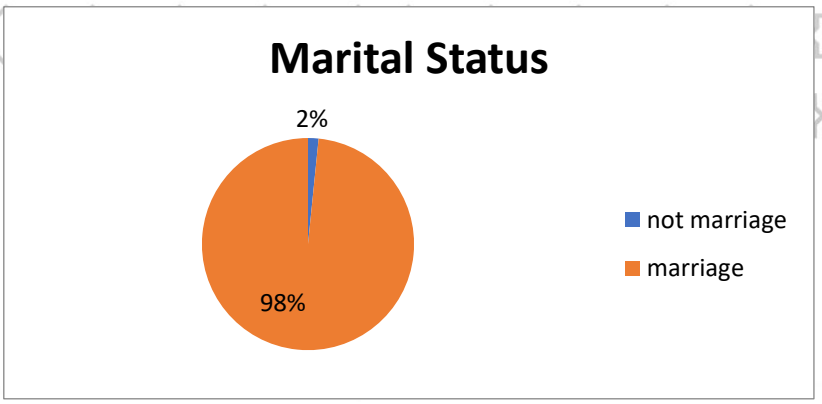
RESULT AND DISCUSSION

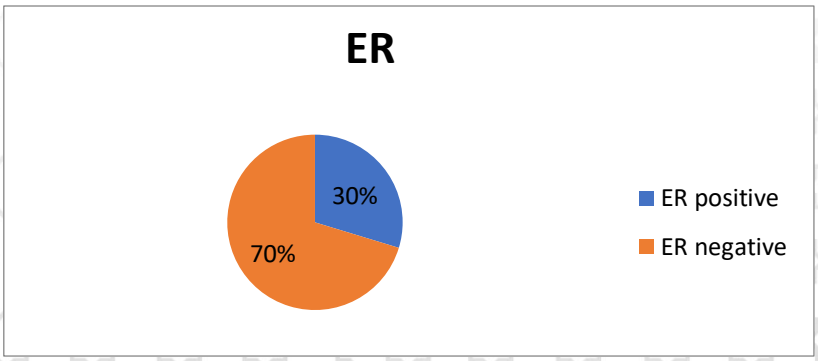
This research was conducted from October to November 2017 at the Anatomy Pathology Laboratory and the medical record section from a hospital. The results of the study are described as follows:

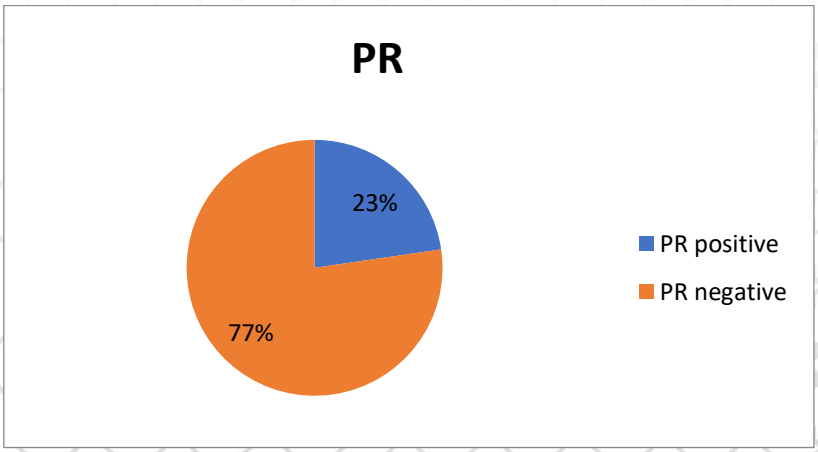





WORK STATUS
6%
9%
27%
58%
Housewife
Entrepreneur
Private employee
Government employee


Marital Status
2%
98%
not marriage
marriage


ER
30%
70%
ER positive
ER negative



This Table provides the data that the patients diagnosed with the most invasive breast carcinoma of the no special type (NST) were those who had the age> 40 years as many as 92 samples (71.9%). This result is in accordance with Ening's study (2015) which stated that the highest incidence of breast cancer based on age was patients> 40 years as many as 138 cases (83.1%). Whereas at the age of ≤ 40 years there were only 28 cases or 16.9%) (Ening and widiana, 2015).

The highest sample was high school education with 52 samples (40.6%). This is in accordance with the research of Dewi (2015) which states that breast cancer patients from the case group have the highest level of high school education or equal to 35.6%. The largest sample also works as a housewife with a frequency of 75 samples (58.6%). These results are in accordance with previous studies that most cases of this patient work as housewives (75.6%) (Dewi and Hendrati, 2015). The existing sample with the status of marriage is the largest sample, 126 samples (98.4%). In accordance with other research which stated that the sample with the status of marriage (marriage) experienced more breast cancer incidence (45.83%) than the unmarried one(30.18%) (Mayasari, 2013).

Based on the results of the ER, the sample that had a negative ER result was the highest sample of 90 (70.3%), while the positive ER frequency was 38 samples (29.7%). These results is in accordance with Khambri (2015) which stated that the negative ER results were 53.3% while the positive ER was 46.7% (Khambri et al., 2015). Based on the results of PR, the sample had a negative PR frequency of 99 (77.3%), and a positive PR frequency of 29 samples (22.7%). This result is also in accordance with the research conducted by Khambri (2015) which stated that the PR results were negative as much as 63.3% while positive PR was 36.7% (Khambri et al., 2015).

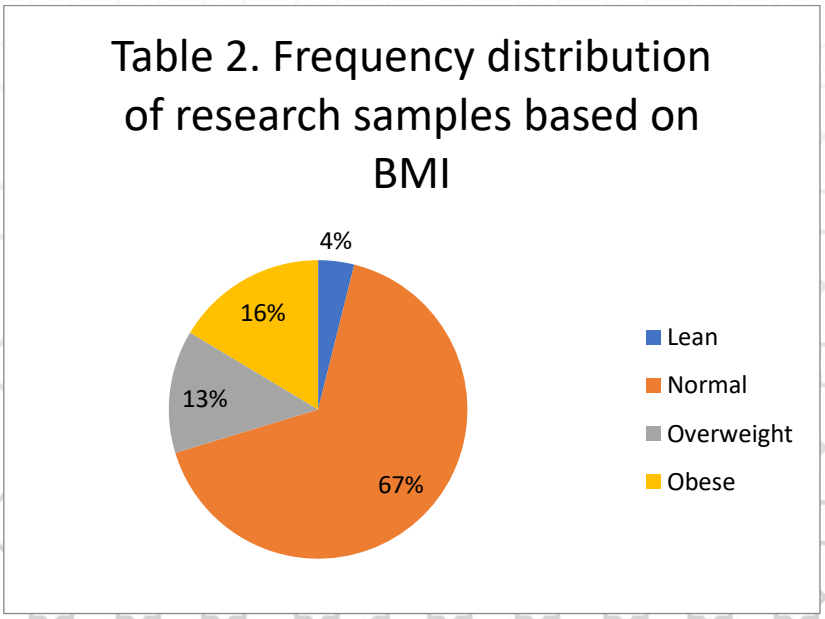


Table 2 provides distribution data that the sample with a normal BMI has the highest frequency of 66.4% (85 samples). These results are consistent with the research conducted by Sugiritama (2015) which states that BMI based on sex shows that 48% of women have normal BMI, 22% are obese, 16% are over weight and 14% are thin (Sugiritama et al., 2015).

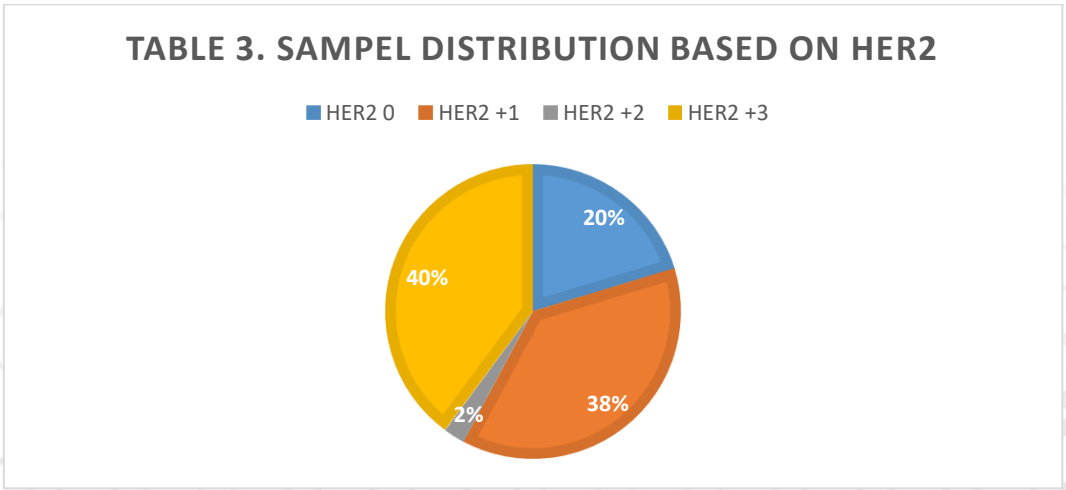


Table 3 provides data on the results of the most immunohistochemical examination, which is HER2+3 is 39.8% (51 samples). These results are in accordance with the research of Mukti (2016) which states that the distribution of HER2 to the clinical stage status of TNM in breast cancer patients shows the results of HER2+3 in 20 cases at the clinical stage of TNM II, 21 cases of clinical stage III and 3 cases of clinical stage IV (Mukti et al., 2016).

Table 4. Gamma Correlation Test Results Relationship between BMI and HER2 in Invasive breast carcinoma of No Special Type (NST)

BMI	IHK Status				Correlation coefisient (r)	P value
	HER2 0	HER2 +1	HER2 +2	HER2 +3		
Thin	1 (20,0)	2 (40,0)	0 (0,0)	2 (40,0)	-0,069	0,590
Normal	19 (22,4)	27 (31,8)	2 (2,4)	37 (43,5)		
Fat	3 (17,6)	8 (47,1)	0 (0,0)	6 (35,3)		
Obese	3 (14,3)	11 (52,4)	1 (4,8)	6 (28,6)		
Total	26 (20,3)	48 (37,5)	3 (2,3)	51 (39,8)		

Hypothesis testing in this study can be seen in table 4 which shows the results of the Gamma correlation test. The value of p = 0.590 (p> 0.05) shows that the correlation between BMI and the molecular phenotype of HER2 is not significant with statistical "moderate" strength. The value of r = -0.069 shows the direction of the negative correlation which means that the higher the BMI with the lower HER2 results or it can be concluded that the obese group (BMI> 27kg / m2) is associated with a low or negative HER2 score (HER2 0 and HER2 +1).

The results of this study are consistent with previous studies conducted by Jiralerspong (2016) which state that the relationship between BMI and HER2+ in breast cancer is unclear. Studies conducted on 1,250 patients recorded a BMI> 30kg / m2 (obesity) with ER- and HER2+ which caused poor output (HR, 1.79; 95% CI, 1.03-3.10) and distant metastases (HR,2.03; 95% CI, 1.13-3.63) compared to patients who have a normal BMI. There was no significant relationship between obesity in patients with ER+ and HER2+. These results indicate that no clear differences were found regarding BMI-based survival in patients with HER2 +. The relationship of obesity may be more related to patients not receiving trastuzumab therapy, a therapy indicated for patients with early-stage breast cancer with HER2 overexpression. Larger studies are needed to determine the association of BMI with survival in patients with HER2+ taking into account ER status and whether or not the effect to be produced is affected by trastuzumab therapy or other HER2 therapy (Jiralerspong and Goodwin, 2016).

Studies conducted by Crozier (2013) also state that there is a tendency (although not statistically significant) for most obese women to have negative hormonal receptors compared to non-obese women. Patients aged> 50 years are more likely to be overweight or obese with HER2+ status and tend to have larger tumors. Women with normal BMI and obesity tend to have positive lymph nodes. There is a statistically significant correlation between increased BMI and worse clinical outcomes with HER2+ in early stage breast cancer patients due to the influence of therapy. Based on body weight, obese patients in this cohort study were more likely to be postmenopausal. This result is in accordance with many national and international studies which state that women with early stages of breast cancer have a positive hormonal receptor (Crozier et al., 2013).

CONCLUSION

The results of the study concluded that there was a correlation between higher BMI and lower HER2 results with moderate correlation strength (p = 0.590) in patients with Invasive breast carcinoma of No Special Type (NST). This study was a preliminary study. by taking into account other factors such as macroscopic size of tissue tumours, diet of patients before and after being diagnosed with breast cancer, history of first degree relatives and history of hormonal contraceptive use from patients.

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ICPU_2019_023

Degranulation Inhibition of Mastocyte Cells and Release of Histamine Water Extract Of *Brugmansia suaveolens*(Bercht&Presl.)

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Abstract

Background: *Brugmansia suaveolens* leaves have been widely used in traditional medicine as an asthma drug and have been scientifically proven. Some studies show that this plant has activity as a bronchodilator, anti-inflammatory, and can inhibit the occurrence of active cutaneous anaphylactic reactions. This study aims to determine the mechanism of action of *Brugmansia suaveolens* leaves extract on allergic responses in vitro method, namely degranulation inhibition and histamine release from rat mastocyte cells of the Wistar strain.
Methods: Rat peritoneum is induced intraperitoneally with Hank solution, to obtain a mastocyte suspension. This Research divided into 6 groups, consisting of normal groups, Pain control group induced vancomycin 10mM, the comparison group given 2% sodium cromoglycate, and 3 groups of *Brugmansia suaveolens* leaves water extract concentrations of 10, 100 and 1000 µg / mL, which saw with hemocytometer, and gave ortho-toluidine dye and method histamine measurements were carried out using UV-visible spectrophotometry.
Results: *Brugmansia suaveolens* leaves water extract with a concentration of 10 µg / mL having the most significant ability to inhibit degranulation of mastocyte cells with 24.4% percent degranulation compared with pain control. The release of histamine mastocyte suspension the test group of *Brugmansia suaveolens* leaves water extract with a concentration of 10 µg/ mL showed the smallest histamine release with a liberation percentage 1071.92%.

Conclusion: The extract of *Brugmansia suaveolens* leaves water extract concentration of 10 µg/ mL had the smallest percent of degranulation and release of histamine and differed statistically significantly by t-student test at $p < 0.05$.

Keywords: *Brugmansia suaveolens*, Degranulation of mastocyte cells, Release of histamine, Liberation of histamine

INTRODUCTION

Mastocyte cells are granules that are rich in histamine and heparin. Mastocytes occur between mucosal tissues and membranes, these cells play a role in the immune system that fights pathogens, cause injury and also play a role in allergic reactions and anaphylaxis. Mastocytes are found in almost all tissues that surround blood vessels, skin, nerves, mucosa of the lungs and digestive tract and are also present in the nose.

When these cells are activated in the presence of antigens, the mastocytes quickly release granules such as histamine, leukotrienes, prostaglandins. These reactions cause enlargement of blood vessels and symptoms of inflammation.

One of the traditional plants that are widely used for treatment and has been scientifically proven in vivo to be used to treat anti-inflammatory is *Brugmansia suaveolens*. In general, *Brugmansia suaveolens* is rarely used because the benefits of treatment are still unknown. However, it turned out that research had been done on Brugmansia suaveolens leaves in vivo and proved to have efficacy inhibiting active cutaneous anaphylactic reactions for allergies, asthma, and analgesics. It is what makes researchers re-use *Brugmansia suaveolens* plants but with different methods, namely in vitro to see further the efficacy of *Brugmansia sueveolens* in treating allergies with different mechanisms, considering the use of anti-allergic drugs is quite widely used in Indonesia.

This study aims to determine the mechanism of action of *Brugmansia suaveolens* leaves extract on allergic reactions in vitro method, namely degranulation inhibition and histamine release from rat mastocyte cells of the Wistar strain.

METHODS

Materials

Brugmansia suaveloens was purchased from Center for Research and Development of Medicinal Plant Manoko lembang, Indonesia. The plant was taxonomically identified in Department Of Biology, School of Biological Sciences and Technology, Bandung Institute Of Technology, Indonesia. Vancomycin(Vancep® Injection) and Cromoglycate Sodium (Conver ® Eye Drops) were obtained from Pharmacy in Cimahi, Indonesia.

Animals

Thirty healthy female Wistar rats aged 8-10 weeks (200-225 gram of body weight) were purchased from Life Sciences Center, Bandung Institute Of Technology, Bandung, Indonesia. Before starting this study, the protocol was approved by the Ethics Committee, Faculty Of Pharmacy, Jenderal Achmad Yani University. The Rats were acclimatised for seven days in

the laboratory and maintained under standard laboratory conditions with dark and light cycles. Standard feed and drink were given ad libitium.

Extraction of *Brugmansia suaveolens*

Brugmansia suaveolens water extract is made using the boiling method, which is by boiling *Brugmansia suaveolens* leaves with distilled water at 100°C for 15 minutes for one boiling. Boiling is done as much as three times repetition, the liquid extract obtained is concentrated by evaporating using an enamel pan to obtain a concentrated extract. The concentrated extract obtained was dried using a water bath and in an oven, at a temperature of 60 ° C until the extract dried. Furthermore, dry extracts were made into three concentrations of 10 ppm, 100 ppm, and 1000 ppm which were dissolved in distilled water (3)

Phytochemical Screening of *Brugmansia suaveolens* Extract

This assay followed the standard methods described from book general standard parameters of medicinal plant extracts published by the Indonesian Ministry of Health. (4)

Testing of Inhibitory Activity of Mastocyte Cell Degranulation

Testing of mastocyte cell degranulation inhibition was carried out based on the modified Norton method. Experimental animals used by Wistar strain rats fasted for 12 hours. Fasted animals were injected with 5ml of Hank solution intraperitoneally then massaged for 2 minutes. After the massage, intraperitoneal fluid is taken using a syringe. In 6 groups divided into mastocyte suspensions containing:

1. The normal group: mastocyte suspension 10 µl + 20 µl Hank solution
2. The pain control group: mastocyte suspension 10 µl added vancomycin 10 µl + 10 µl Hank solution
3. Comparison group: mastocyte suspension 10 µl added vancomycin 10 µl + 2% sodium cromoglycate as much as 10 µl
4. 10µg / ml concentration group: mastocyte suspension 10 µl added vancomycin 10 µl + 10 µl *Brugmansia suaveolens* extract
5. 100µg / ml concentration group: mastocyte suspension 10 µl added vancomycin 10 µl + 10 µl *Brugmansia suaveolens* extract
6. Concentration group 1000µg / ml: mastocyte suspension 10 µl added vancomycin 10 µl + 10 µl *Brugmansia suaveolens* extract

The mixture obtained was incubated for 30 minutes at 37 ° C. After incubation, 10 µl of 0.1% ortho toluidine blue solution is stirred slowly. The mixture is dripped into the hemocytometer and counted the number of mastocytes not degranulated under a microscope with 400x enlargement. (5,6)

Making Histamine Calibration Curves

Weighed with pure 30 mg histamine and dissolved in distilled water up to 10 ml, the main liquor was obtained with a concentration of 3000 ppm. The main liquor is diluted to 300 ppm. 300 ppm solution became the second main liquor and was diluted into several concentrations of 6 µg / ml, 18 µg / ml, 36 µg / ml, 60 µg / ml, 72 µg / ml and 96 µg / ml.(7)

Preparation of p-benzyldiazonium sulfonate reagent

Mixed with 15 mL 0.9% sulfanilic acid in concentrated HCl and 15 ml 5% NaNO2 then soaked in ice water for 5 minutes. The total of 6 ml of 5% NaNO2 solution was added and added to cold distilled water to 500ml. The solution was left for 5 minutes then the reagent was stored in an ice bath for 15 minutes. (7)

Testing the Amount of Histamine Released

The Release of histamine the mastocyte suspension is divided into six groups, namely:

1. Normal group: mastocyte suspension one µl added with two µl distilled water
2. Pain control group: mastocyte suspension one µl added vancomycin10 mM one µl and distilled water one µl
3. Comparison group: mastocyte suspension one µl added vancomycin 10 mM one µl and 2% sodium cromoglycate as much as one µl
4. Concentration group 10 µg / ml: mastocyte suspension one µl added one µl of vancomycin 10 mM and 1 µl of an extract of Brugmansia suaveolens10 µg / ml
5. Concentration group 100 µg / ml: mastocyte suspension one µl added vancomycin 10 mM one µl and extract of Brugmansia suaveolens100 µg / ml one µl
6. Concentration group 1000 µg / ml: mastocyte suspension one µl added vancomycin 10 mM one µl and extract of Brugmansia suaveolens 1000 µg / ml one µl

The obtaining mixture was incubated at 37oC for 30 minutes, after which 600µl NaCl was added and centrifuged at a rate of 3100 rpm for 10 minutes. The solution obtained was taken as clear as 0.5ml and added with a solution of 1ml sulfanilic acid and 2.5ml Na2CO3 solution. The obtaining mixture was allowed to stand for 5 minutes then the absorption was measured by UV-visible spectrophotometry at a wavelength of 427.5 nm.(7)

Statistical Analysis

The obtained data were expressed as the mean+standard deviation and the differences between multiple groups were calculated using SPSS for the window with Student – t-Test, and a *p*-value of 0.05.

RESULTS

Phytochemical Screening

Phytochemical screening of Brugmansia suaveolens water extract revealed positive results for alkaloids, flavonoids, saponin, tannins, polyphenol, steroids triterpenoid, and monoterpenes sesquiterpenes. (8)

Testing of Inhibitory Activity of Mastocyte Cell Degranulation

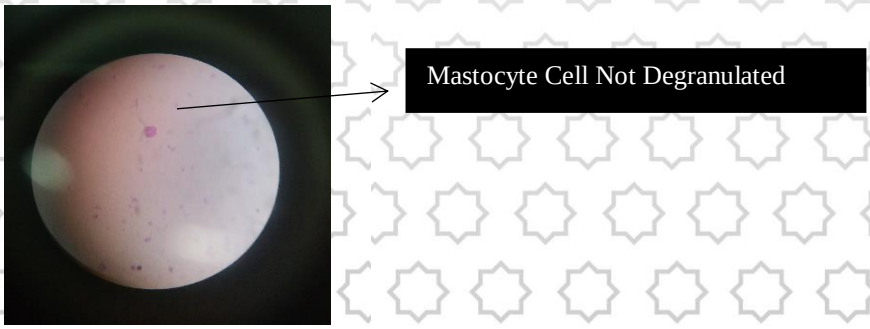

Figure 1 Mastocyte Cell Not Degranulated

Mastocyte cells not degranulated show blue-purple to red-purple.

Table 1. Number of Mastocytes Not Degranulated

Number Of Mastocyte Not Degranulated (cell/mm3) (n)					
Animal Group					
Normal	Pain Control (Vancomycin)	Comparison (Sodium cromoglycate)	BS Water Extract Concentration 10 µg/ml	BS Water Extract Concentration 100 µg/ml	BS Water Extract Concentration 1000 µg/ml
67.5	12.5[a]	47.5 [a,b]	52.5 [a,b.c]	45.0 [a,b.c]	25.0 [a,b]
65.0	20.0 [a]	40.0 [a,b]	57.5 [a,b.c]	40.0 [a,b.c]	30.0 [a,b]
75.0	10.0[a]	55.0 [a,b]	50.0 [a,b.c]	37.5 [a,b.c]	22.5 [a,b]
80.0	17.5 [a]	67.5 [a,b]	47.5 [a,b.c]	32.5 [a,b.c]	35.0 [a,b]
70.0	7.5 [a]	42.5 [a,b]	60.0 [a,b.c]	42.5 [a,b.c]	27.5 [a,b]
71.5±6.0	13.5±5.1	50.5±11.0	53.5±5.1	39.5±4.8	28.0±4.8

BS = *Brugmansia suaveolens*
n = 5
a = Significant difference compared to normal group($p<0.05$)
b = Significant difference compared to pain control($p<0.05$)
c = Not Significant difference compared to comparison group($p<0.05$)

In the pain control group, the least number of mastocytes cells were lacking. This suggests that induction with vancomycin can cause degranulation of mastocyte cells.

Table 2. Percent of Mastocyte Degranulation

Percent Of Mastocyte Degranulation(%)					
Animal Group					
Normal	Pain Control (Vancomycin)	Comparison (Sodium cromoglycate)	BS Water Extract Concentration 10 µg/ml	BS Water Extract Concentration 100 µg/ml	BS Water Extract Concentration 1000 µg/ml
0	81.48	29.63	22.22	33.33	62.96
0	69.23	38.46	11.54	38.46	53.85
0	86.67	26.67	33.33	50.00	70.00
0	78.13	15.63	40.63	59.38	56.25
0	89.29	39.28	14.28	39.29	60.71
0	80.96 ±7.87	29.93 ±9.68	24.40 ±12.40	44.09 ±10.47	60.75±6.29

% degranulation = $\frac{(n\ normal)-(n\ treatment])}{(n\ normal)} x100\%$ (See table 1)

n =5

The group of Brugmansia suaveolens leaves water extract ten µg/ml concentrations had the least percent of degranulation contained in the pain control group.

Testing the Amount of Histamine Released

Table 3. The histamine concentration released by mastocyte cells suspension rat

	Histamine Concentration (µg/ml)					
Measurement	Normal	Pain Control (Vancomycin)	Comparison (Sodium cromoglycate)	BS Water Extract Concentration 10 µg/ml	BS Water Extract Concentration 100 µg/ml	BS Water Extract Concentration 1000 µg/ml
1	5.55	97.84	27.31 [a]	37.38 [a,b]	57.00 [a,b]	86.85
2	2.57	100.44	30.05 [a]	40.28 [a,b]	57.53 [a,b]	99.29
3	3.03	74.40	32.04 [a]	38.68 [a,b]	40.66 [a,b]	97.15
Mean±SD	3.72± 1.6	90.89± 14.33	29.80± 2.37	38.78± 1.45	51.73± 9.59	94.43± 6.65

n=3
a = Significant difference compared to pain control($p<0.05$)
b = Significant difference compared to comparison group($p<0.05$)

Group of Brugmansia suaveolens leaves water extract concentration 1000 µg/ml has the most significant amount of histamine released among all groups.

Table 4. Liberation percent of histamine in mastocyte cells suspension rat

	Liberation Percent (µg/ml)				
Measurement	Pain Control (Vancomycin)	Comparison (Sodium cromoglycate)	BS Water Extract Concentration 10 µg/ml	BS Water Extract Concentration 100 µg/ml	BS Water Extract Concentration 1000 µg/ml
1	1662.99	392.02	573,59	927.09	1464.92
2	3804.15	1068.25	1465.87	2136.49	3759.64
3	2355.16	957.18	1176.32	1241.81	3105.79
Mean±SD	2607.43± 1092.64	805.81± 362.63	1071.92± 455.21	1435.13± 627.45	2776.78± 1182.21

% Histamine Liberation = $\frac{(c\ treatment)-(c\ Normal)}{(c\ normal)} x100\%$ (See Table 3)

The higher percentage of histamine liberation is shown by the group of Brugmansia suaveolens leaves water extract, it means that the higher concentration of Brugmansia suaveolens leaves water extract shows the higher histamine release from mastocyte cells.

Table 5. Degranulation percent and liberation of histamine results

Animal Group	Degranulation Percent	Histamine Liberation Percent
Pain Control	80.96±7.87	2607.43±1092.64
Comparison	29.93±9.68	805,81±362.63
BS Water Extract Concentration 10 µg/ml	24.40±12.40	1071.92±455.21
BS Water Extract Concentration 100 µg/ml	44.09±10.47	1435.13±627.45
BS Water Extract Concentration 1000 µg/ml	60.75±6.29	2776.78±1182.21

DISCUSSION

Before the test was carried out the rats were fastened for 18 hours, the analysis was carried out using a modified method by taking rat mastocytes obtained by injecting Hank's solution into the abdominal cavity(5). This was intended to suspended mastocyte cells in the rat abdominal cavity so that they were easily obtained. The suspension of mastocyte cells that have been obtained were divided into normal groups, pain control group (vancomycin ten mM), comparison group (sodium cromoglycate 2%), Brugmansia Sueveolens leaves water extract

concentration group 10 µg / ml, 100 µg / ml and 1000 µg / ml. The comparison used is sodium cromoglycate because it can reduce allergies by stabilizing mastocyte cells by inhibiting calcium ion influx so that histamine release does not occur. (9)

The suspension of mastocytes that have been mixed with the test was added with 0.1% ortho toluidine blue dye as much as 10 µl, which aims to give colour to the undegranulated cells, the undegranulated cells showed blue-purple to red-purple, then the obtained mixture was incubated for 30 minutes at 37 ° C to give a chance for IgE in the serum to sensitize or attach itself to mastocyte cells. (10)

Degranulation inhibition can be said to be successful if the percent degranulation is not greater than 30%, if the percentage of more than 30% indicates the presence of IgE interaction with the antigen which causes the release of granules from mastocyte cells. If mastocyte cells release granules, they can cause enlargement of blood vessels and symptoms of inflammation. (5)

In each group there are different treatments, treatment without vancomycin and therapy with vancomycin can cause degranulation as a result of treatment techniques. In the pain group showed 80.96% percent degranulation and 2607.43 percent histamine liberation, this suggests vancomycin was able to degranulate, in other words, the method or induction of degranulation was successful. When compared to pain control, there was 29.93% degranulation suppression and 805.82 percent liberation histamine. This indicates that the method used is valid to see the effect on degranulation and histamine liberation. In the test group, there were three groups, namely the concentration of 10 µg / ml, 100 µg / ml and 1000 µg / ml. From the results obtained it can be seen that the concentration of Brugmansia suaveolens leaves water extract concentration 10 µg / ml can inhibit degranulation to 24.4% and has a small liberation percentage of 1071.92% when compared with the concentration group of 100 µg / ml and 1000 µg / ml. Statistical results of Brugmansia suaveolens leaves water extract dose of 10 µg / ml, and the comparison group showed no difference ($p>0.05$). This showed an effect that was comparable to the comparison of sodium cromoglycate even though degranulation and liberation of histamine were still above the control of pain. The fewer cells are degranulated, the fewer histamine is released.

CONCLUSION

This study showed that induction using vancomycin succeeded in rendering cells degranulated with percent degranulation of 80.96%, and when compared with comparison it was successful in reducing degranulation to 29.93%. Brugmansia suaveolens leaves water extract which successfully inhibited degranulation was a concentration of 10 µg / ml with per cent degranulation of 24.4% and per cent liberation of histamine of 1071.93%. In vitro showed that Brugmansia suaveolens leaves water extract can suppress allergic reactions.

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