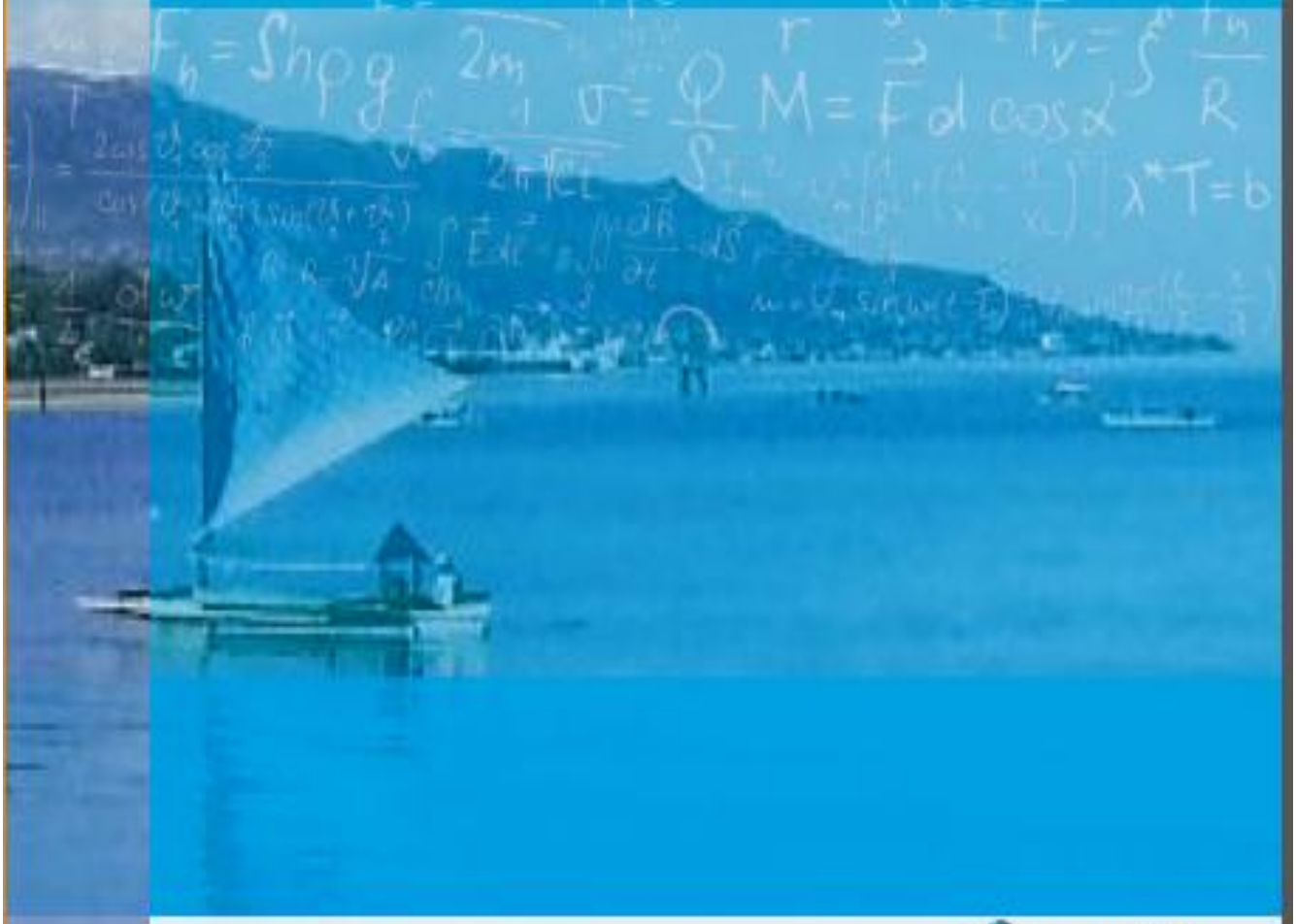
Basic Science
for Sustainable Marine Development

PROCEEDING
INTERNATIONAL SEMINAR 2015

Ambon, 3-4 June 2015



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Faculty of Mathematics and Natural Sciences
Pattimura University

PROCEEDINGS

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Welcoming Address by The Organizing Committee

The honorable, the rector of Pattimura University

The honorable, the vice rector of academic affair, Pattimura University

The honorable, the vice rector of administration and financial affair, Pattimura University

The honorable, the vice rector of planning, cooperation and information affair, Pattimura University

The honorable, all the deans in Pattimura University

The honorable, the key note speakers and other guests.

We have to thank The Almighty God for the blessings that allow this International seminar can be held today. This is the first seminar about MIPA Science in which the Faculty of MIPA Pattimura University becomes the host. The seminar under the title Basic Science for Sustainable Marine Development will be carried out on 3 June 2015 at Rectorate Building, the second floor. There are 250 participants from lecturers, research institute, students, and also there are 34 papers will be presented.

This International seminar is supported by the amazing people who always give financial as well as moral supports. My special thanks refer to the rector of Pattimura University, Prof. Dr. Thomas Pentury, M.Si, and the Dean of MIPA Faculty, Prof. Dr. Pieter Kakissina, M. Si. I also would like to express my deepest gratitude to Dr. Kotaro Ichikawa, the director of CSEAS Kyoto University, Prof. Bohari M. Yamin, University of Kebangsaan Malaysia, Prof. Dr. Budi Nurani Ruchjana (Prisident of Indonesian Mathematical Society/Indo-MS), Dr. Ir. A. Syailatua, M.Sc (Director of LIPI Ambon), and Hendry Ishak Elim, PhD as the key note speakers. We expect that this international seminar can give valuable information and contribution especially in developing basic science for sustainable marine development in the future.

Last but not least, we realize that as human we have weaknesses in holding this seminar, but personally I believe that there are pearls behind this seminar. Thank you very much.

Chairman

Dr. Netty Siahaya, M.Si.

Opening Remarks
By Dean of Mathematic and Natural Science Faculty

I express my deepest gratitude to The Almighty God for every single blessing He provides us especially in the process of holding the seminar until publishing the proceeding of International Seminar in celebrating the 17th anniversary of MIPA Faculty, Pattimura University. The theme of the anniversary is under the title Basic Science for Sustainable Marine Development. The reason of choosing this theme is that Maluku is one of five areas in Techno Park Marine in Indonesia. Furthermore, it is expected that this development can be means where the process of innovation, it is the conversion of science and technology into economic value can be worthwhile for public welfare especially coastal communities.

Having the second big variety of biological resources in the world, Indonesia is rich of its marine flora and fauna. These potential resources can be treated as high value products that demand by international market. Basic science of MIPA plays important role in developing the management of sustainable marine biological resources.

The scientific articles in this proceeding are the results of research and they are analyzed scientifically. It is expected that this proceeding can be valuable information in terms of developing science and technology for public welfare, especially people in Maluku.

My special thanks refer to all researchers and reviewers for your brilliant ideas in completing and publishing this proceeding. I also would like to express my gratefulness to the dies committee-anniversary of MIPA Faculty for your creativity and hard working in finishing this proceeding, God Bless you all.

Dean of Mathematic and Natural Science Faculty

Prof. Dr. Pieter Kakisina, M.Si.

Contents

Effect Of Ethanol Leaf Extract Gambir Laut (*Clerodendrum inerme* L. Gaertn) Malformations On Motion To External Equipment Fetal Development Mice (*Mus musculus*)

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ABSTRACT

Gambir laut (*Clerodendrum inerme* L. Gaertn) is one of the plants that contain several chemical compounds such as flavonoids, saponins, tannins, triterpenoids and glycosides in which the group of chemical compounds can potentially affect the fetal development of mice (*Mus musculus*). This study aimed to determine the effect of ethanol extract of leaves of gambir laut (*Clerodendrum inerme* L. Gaertn) against external malformations in fetal development locomotor mice (*Mus musculus*). Pregnant mice were divided into four treatment groups. Each group was given the extract at a dose of 0, 350, 700 and 1400 mg / g BW on gestation day 6 to 15 and on day 18 mice were dissected parent to external malformations observed in locomotor of the fetus. The results showed the ethanol extract of the leaves of Gambir Laut increase the number of external malformations in locomotor from fetal mice. For the upper extremities percentage of external malformations appear at a dose of 350 mg/g of 22.99%, a dose of 700 mg/g of 77.78% and a dose of 1400 mg/g of 83.33%. For the lower extremities percentage of external malformations that appear on the dose of 350 mg/g of 30.54%, a dose of 700 mg/g of 81.48% and a dose of 1400 mg/g BB of 76.67%.

Keywords: *Gambir Laut (Clerodendrum inerme L. Gaertn),* Ethanol Extract malformations, extrimity.

INTRODUCTION

Gambir laut (*Clerodendron inerme* L.) is a plant species that is commonly used by traditional communities and empirically believed to cure several diseases. Maluku (especially the State Tulehu) gambir laut used to treat malaria and infusa have antimalarial effects by inhibiting the growth of *Plasmodium berghei* (Latuihamallo, 2012). Inhibition of the growth of *Plasmodium berghei* allegedly due to the effects of some chemical compounds contained therein.

The chemicals contained in gambir laut are flavonoids, saponins, tannins, triterpenoids and glycosides in which classes of active compounds are potentially as antifertilitas (Winarno and Dian, 1997). As antifertilitas, Saponins and flavonoids are compounds that are estrogenic (resemblance to estrogen hormone) in the body where when estrogen levels are high due to external factors can increase the sensitivity of the uterus to oxytocin so the motility of the uterus and genital tract female increased and resulted in disruption of

fertilization and embryo development because the uterus is not able to supply enough nutrients for embryonic development (Madyawati et al, 2003 in Rusmiati, 2009)

Besides mentioning triterpenoids and saponins in plants can affect the permeability of cell membranes (Geisert et al, 1997; Mitaine et al., 2001 in Wurlina, 2006). Permeability of cell membranes of eggs and embryos are closely related to the growth and development of the fetus (cleavage) that can cause shrinkage membrane so that the integrity of the membrane will be decreased. Ethanol extract given to rats during organogenesis parent cause fetal morphological abnormalities in the form of a dwarf, hemorrhage, and deformed legs bent (talipes) (Setyawati, 2009).

Based on the description of the research aims to determine the effect of ethanol extract from the leaves of gabir laut (*Clerodendron inerme* L.) against external malformations locomotor on fetal development of mice (*Mus musculus*).

METHODS

This study with four treatments as follows: control group; Gambir laut extract treatment group 350 mg / gr BW, 700 mg / gr BW, and 1400 mg / gr BW.

Extraction

Making the ethanol extract of leaves of gambir laut performed by using the method according to the Christian, (2013) as follows: gambir laut leaves are dried and pulverized. Taken leaf powder gambir laut much as 100 grams and then inserted into two erlenmeyer 1000 ml sizes. After that put 70% ethanol 500 ml in each flask and allowed to stand for 24 hours. After 24 hours, the solution was filtered using Whatman filter paper to obtain a liquid extract of gambir laut and inserted into the Erlenmeyer with a size of 500 ml. Extraction residue is repeated until a clear colorless solution indicating that all metabolic has been filtered. Liquid extract from the leaves of gambir laut ethanol are then collected and evaporated at a temperature of 400 C to obtain a concentrated extract ethanol. Ethanol extract which has been concentrated further weighed and diluted with distilled water according to the required concentration.

Preparation of Mice

On day 1 given PMSG (pregnant mare serum gonadotropin) is intraperitoneal as much as 0.01 ml and on day 3 was given HCG (human chorionic gonadotropin) as much as 0.01 ml. Female mice that had been given PMSG and HCG, mated with male mice collected manner in a cage by using one method of mating. The next day was observed for the vaginal plug.

Treatment

The treatment was done on day 6 to day 15 of gestation (organogenesis period). Ethanol extract of leaves of gambir laut with a dose of 0.2 ml. On the 18th day of gestation, the mother mice nekropsis, dissected, and the uterus is taken to remove the fetus. The parameters observed on the fetus is malformed external upper and lower limbs that appear.

RESULTS AND DISCUSSION

In the control group (Figure 1A) does not occur malformations externally on locomotor fetal mice, whereas the treatment group a dose of 350 mg / g mice (Figure 3B) can be shown that the limb or limbs on fetal mice still perfectly shaped, which if observed morphologically, from both upper limbs in the fetal mice was shaped upright bent forward and not either into, or out as shown in fetal mice of the treatment group dose of 1400 mg / g mice (Figure 1D). But unlike the limb or lower limb shape is more likely to be open when compared with the control group. For fetal mice from dose group of 700 mg / g mice have abnormalities in the form of talipes extremities above, where the position of the legs on the right, twisted towards the bottom when compared with the legs to the left (Figure 1C) and the latter to the fetus from the group dose 1400 mg / g BB mice (Figure 1D) suffered structural defects on the upper and lower extremities when compared with the control group. Yangv malformations occur forms can be seen in Figure 1.

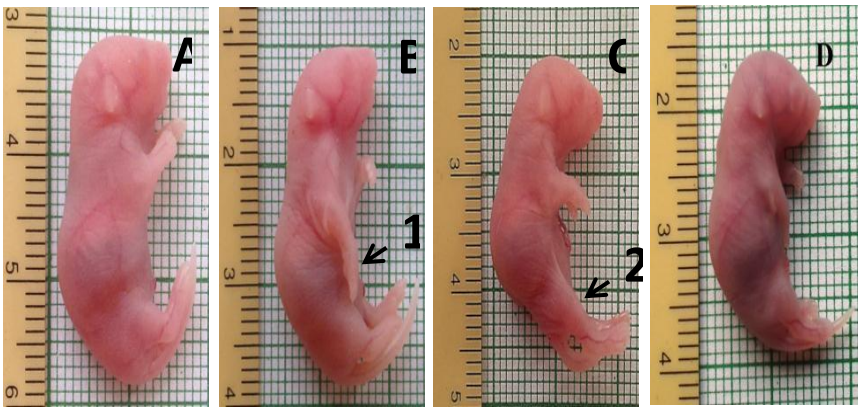

Figure 1. Malformation Fetus mice (Mus musculus L.) A = The control group, B= Treated extract 350 mg / g B, C = Treatment extract 700 mg / g, D= Treatment extract 1400 mg / g. (1) upper limb malformations; (2) lower extremity malformations.

For the percentage of fetal malformations in mice externally locomotor at the upper extremities and the lower extremities can be seen in Figure 1. Based on the diagram below that divides the external malformations in mice's locomotor ektrimitas upper and lower extremities, it can be seen for the control group did not happen malformations, whereas the dose group of 350 mg / g body weight and dose group of 700 mg / g can be seen the percentage of malformations external to the locomotor fetal mice to the lower extremities higher than at the top, but the group dose 1400 mg / g is more likely to be found malformations locomotor for upper extremities higher than the lower extremities.

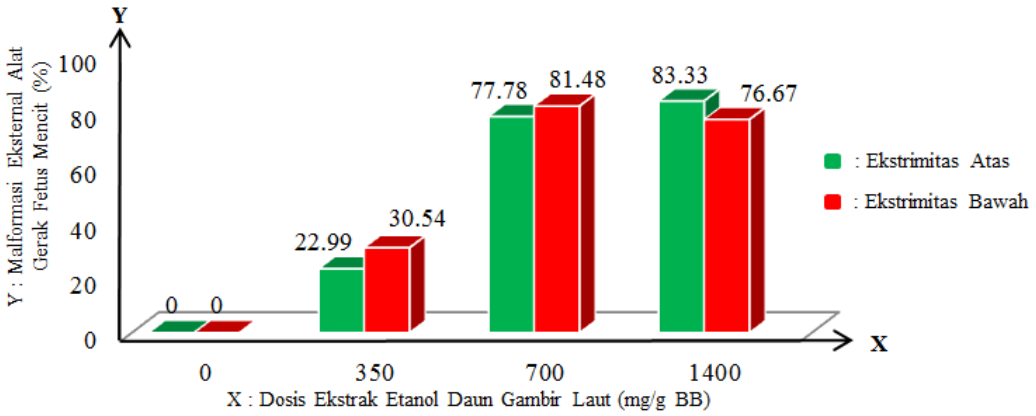

Figure 2. Diagram of external malformations locomotor percentage of fetal mice for the extremities above and ekstrimitasbawah in each treatment group.

Ethanol extract from the leaves of gambir laut on the parent murine embryonic development during gestation day 6 to up to 15 (organogenesis period), can cause malformations (structural defects) on the part of members of the motion (extremities). The occurrence of malformations (structural defects) in the limbs of mice fetus is caused because the content of active compound contained in the ethanol extract of leaves of gambir laut. Disability structural in the limbs of the fetus mice were found in accordance with the opinion of Sundaryono (2011), which states that the period susceptible parent mice against outside influences in this case the drugs or chemicals starting from day 7 to 11, but vulnerability in the development of fetal mice locomotor occurred on the day of gestation to 9 to 11. According Prohati (2013), the results of research conducted at the Laboratory of Traditional Medicine Faculty of Pharmacy USU in 2012, declared the sea gambier contains several active compounds such as; triterpenoids, flavonoids, glycosides, saponins and tannins which some of these compounds may be a teratogen.

Flavonoids and saponins contained in the extract Phaleria known to be cytotoxins. because it can cause the death of some of the cells making up the vertebrae, resulting in bone growth rate terkhususnya for limb is not the same so that bones become bent (Widyastuti et al, 2006). Moreover, it can also increase uterine smooth muscle contraction that causes mechanical stress on the embryo which can cause changes in the direction of growth so that the resulting fetus will appear deformed when observed in terms of morphology.

In this study found the morphology of the locomotor top and bottom of the curved fetal mice tend to be shorter with the palm of the bend to the inside, while the normal locomotor top and bottom tend to be longer with both open position gets outside. This is due to the limited space in the parent murine uterus resulting in abnormal development of the fetus in locomotor mice.

In this study also seen the percentage of malformations externally locomotor from fetal mice for the extremities upper and lower extremities found more damage to the lower extremities, however, the increased amount of damage that occurred, when calculated from the difference between each treatment dose, the increase in most steeper actually happened on the upper extremities. According to Sadler (1997), the process of limb development on stage or have characteristics in common with the process of limb development lower limb morphogenesis except under approximately 1 to 2 days after the limbs above

Upper extremity development that occurred earlier than the lower extremities during the period of organogenesis This leads to a percentage increase of the number of external malformations in locomotor higher in upper extremities. It is also stated by Subowo (1992), stating that the femur os humerus, called the lower limb so that ossification or bone growth occurs earlier upper limb than the lower limb. Currently, embryonic development to the stage of bone formation limbs, on the day of gestation to 9 to 11 parent mice, occurred crenation or shrinkage of cells making up the smooth muscle of the uterus, resulting in a narrowing of the space for the fetus in the uterus of mice. Consequently upper extremities were first grown from the bottom will suffer malformations ektrimitas first. This is because the development of the upper extremities earlier coincided with the period of organogenesis, so that the effect of the ethanol extract of the leaves of gambir laut causes uterine smooth muscle contraction will be more impact on upper limb development when compared to the lower extremities.

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