



Histology Structure of Lymph and Uterus of White Rat (*Rattus norvegicus*) Given with *Nanas Bongsai* (*Ananas comosus var. microstachys* L.)

✉Fitmawati¹, Agus Saputra¹, Yohanes², Hilwan Yuda Teruna³, Dimas Pramita Nugraha⁴, Yulis Hamidi⁴

DOI: 10.15294/biosaintifika.v10i1.13596

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Riau Kampus, Indonesia

²Public Health Official, Riau Province, Indonesia

³Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Riau, Indonesia

⁴Department of Medical, Faculty of Mendical, Universitas Riau, Indonesia

History Article

Received 16 November 2017
Approved 25 March 2018
Published 30 April 2018

Keywords

Nanas Bongsai (*Ananas comosus var. microstachys* L.); White rat (*Rattus norvegicus*); Lymph; Uterus

Abstract

Nanas Bongsai (*Ananas comosus var. microstachys* L.) is an amazing herb which is used in traditional medicine by local people of *Muara Lembu* district as a potion to reduce pain while menstruation period for a women. The present work was designed to investigate its probable side effects on the histopathologic changes in lymph and uterus tissues after treated with *A. comosus var. microstachys* L extract in female white rats. This is an experimental research consisted of five treatments and three repetitions. Treatment composed of two controls (P_0 and P_1) and given *Nanas Bongsai* extract with three different dosages. Histology result of lymph show that all treatments given with *Nanas Bongsai* with three different dosages have different diameter average of white pulp and there is no significant changes with normal control. While observation towards histology structure of endometrium thickness with dosage P_1 , dosage P_2 , dosage P_3 is 206.333 ± 33.486 ; 215.667 ± 33.486 ; 197.667 ± 60.871 respectively. The most thin endometrium layer found in treatment with dosage P_3 compared to control P_0 . From this study showed that there is no toxic effect on uterus and lymph function of of *Nanas Bongsai* (*A. comosus var. microstachys* L.) at different doses, so the plant is secure for consumption by the community.

How to Cite

Fitmawati, Saputra, A., Yohanes, Teruna, H. Y., Nugraha, D. P. & Hamidi, Y. (2018). Histology Structure of Lymph and Uterus of White Rat (*Rattus norvegicus*) Given with *Nanas Bongsai* (*Ananas comosus var. microstachys* L.). *Biosaintifika: Journal of Biology & Biology Education*, 10(1), 223-228.

© 2018 Universitas Negeri Semarang

✉ Correspondence Author:

Jl. HR. Soebrantas Km 12.5 Pekanbaru 28293, Riau, Indonesia

E-mail: fitmawati2008@yahoo.com

p-ISSN 2085-191X

e-ISSN 2338-7610

INTRODUCTION

Utilization of medicinal plants has long been known by Indonesians called as *jamu*. Knowledge about utilization of this medicine has inherited from generation to generation both oral and lettering. Utilization of this medicine has been widely used by local people. One noticeable reason to use this plant is high price of synthetic medicine (Cottencin *et al.*, 2014). According to World Conservation Monitoring Center claimed that Indonesia was a region that rich of many medicinal plants. Medicinal plants that have been utilized about 2,518 kinds of medicinal plants (EISAI, 1995). In Indonesia at least 3,000 kinds of medicinal plants have been identified (Zuhud & Hikmat, 1998). Knowledge of herbal medicine and mixed herbal potions originally came from the knowledge of local community gained inadvertently as indigenous knowledge and developed by generations (Fitmawati *et al.* 2017)

In Riau Province, there is a lot of traditional medicinal knowledge specifically in *Muara Lembu* village Singingi Hilir subdistrict, Kuantan Singingi district. The societies have been used to use *Nanas Bonsai* (*Ananas comosus* var. *microstachys* L.) from family Bromeliaceae which used for commercial fruit production in Indonesia as medicinal plants. This plant was believed by local community able to reduce pain during menstruation period and have been consumed for generations. Empirical verification is highly required in order to improve this plant. According to Ekor (2014) the accuracy of consuming traditional medicine correctly and have been tested both of preclinical and clinic test able to prevent adverse effect from traditional medicine itself.

An effort to commercialization of *jamu* required a standardization and preclinical test towards the simplicial. Simplicial standardization is a whole effort begins with the selection of certain medicinal plants. This is a preclinical test using *Nanas Bonsai* extract for lymph and uterus organs. The lymphatic system may control the ultimate immune response including antigens coming outside from the body (Fitmawati *et al.*, 2017; Liao *et al.*, 2016; Prakash *et al.*, 2013), and plays an important role in homeostasis by controlling extracellular fluid volume and in combating infection (Margaris *et al.*, 2012). The uterus is the major female reproductive organ of mammals, including humans. The female rabbit has a bicornuate duplex uterus. This type of uterus has two separate uteri. Each uterus has its own cervix, and the two cervixes open into a single vagina. Many of fertility problems in mammals

are of uterine origin (Alkareem, 2017).

As this information is traditional and there is a dearth of publications on the effects of this plant on the lymph and uterus tissues, this research was aimed to investigate its probable side effects on the histopathologic changes in lymph and uterus tissues after treated with *Nanas Bonsai* extract in female white rats at different serial dosages. This research supports the importance of knowledge and of comprehensive data from medicinal plants *Nanas Bonsai* that is ancestral heritage community of *Muara Lembu* village Singingi Hilir subdistrict, Kuantan Singingi district, Riau Province.

METHODS

Preparation of the Extract of *Nanas Bonsai*

Extraction method was made by local people habit by making the extraction for 100 ml. *Nanas Bonsai* that already rinsed and squeezed to obtain the water made the extract. Add water gradually, the extract were used for treatments process. This extract only use once in a day for eight days.

In Vivo Assay

This research is an experiment research that conducted in laboratory using 15 female white rats aged 3 months and weighing 160g-200g. This research using completed randomized design with five treatments and three repetitions that consisted of two controls; zero control giving water (P₀), positive control by giving mefenamat acid (P₊) and extract of *Nanas Bonsai* were varied with three different dosages. Treatments were conducted orally once in a day for eight days using 1 ml sput disposable without pin. At 8th day, all white rats were euthanasia using chloroform.

Dosage Determination of Experimental Animal

Dosage determination based on Lawrance & Bacharach (1964) by converting common dosage consume by human (100 ml) with white rats conversion factor 0.018 and obtained conversion factor 1.8 ml/200g BB that have been set as two route dosage given orally. Then the dosages were graded 0.5x, 1x and 1.5. 0.9 ml/200g BB, 1.8 ml/200g BB, 2.7 ml/200g BB respectively.

Making Process of Preserved Preparations

Preparation process of tissue sample at lymph and uterus using paraffin method. The organ were checked and fixed using BNF liquid (*Buffered Neutral Formalin*) 10% for 24 hours. The

next following stage is the organ were cut transverse and arrange in tissue cassette and then soaked in BNF 10% for 15 minutes and rinsed using water. Dehydration process using alcohol 30%, 50%, 70%, 90% and ethanol for 45 minutes and clearing process using xylol I and II for 45 minutes and followed by infiltration using paraffin. Embedding process into mold block and poured using paraffin liquid for a night. After solidified sectioning process using microtom with thickness about 6-7 mikron. The next step is deflation using ethanol I and II, graded alcohol 96%, 80%, dan 70% for two minutes. Staining process including Hematoxylin-Eosin (HE). Observation using microscope and documented.

Data Analysis

Observation towards histology were analysed descriptively. Microscopic data were analysed by observing damaged cells on kidney and liver of white rats by observing five viewing field. Obtained data then analyzed using ANOVA and followed by *Duncant Multiple Range Test* (DMRT) (Assiam *et al.*, 2014). Data analysis for Lymph and uterus histology by scoring the percentage of damage cells based on Baldatina (2008).

$$\text{Percentage of damage cell (\%)} = \frac{\text{Damage cell} \times 100\%}{\text{All cells}}$$

RESULT AND DISCUSSION

Microscopic Observation of Lymph

Lymph is the biggest lymphoid organ in body that design as organ for immune response towards foreign substance that enters to body (Aaspelund *et al.*, 2016; El-Sherief *et al.*, 2017). In this research observation towards microscopic structure changes towards white rats lymph that have been given five treatments entailing control (P_0 and P_+) as well as three different dosage serial of *Nanas Bongsai* extract. One of parameter need to observe changes in diameter size of white pulp in every treatment. The data were presented in Table 1.

Table 1. Diameter average of lymph white pulp of white rat.

Treatment	Diameter Average (μm)
P_0	155.6 \pm 22.517 ^{ab}
P_+	166.333 \pm 15.358 ^b
P_1	136.933 \pm 3.349 ^a
P_2	153.267 \pm 13.927 ^{ab}
P_3	136.067 \pm 3.7 ^a

Notes: P_0 : zero control, P_+ : positive control, P_1 :

Nanas Bongsai Dosage 1, P_2 : *Nanas Bongsai* Dosage 2, P_3 : *Nanas Bongsai* Dosage 3. Numbers followed by different alphabet at same coloumn is significantly different at α 5%

Table 1. shows the average of pulp diameter of white rat lymph after given treatment. Based on the table differences were shown between pulp diameter in every treatment. Based on ANOVA result for white pulp lymph P value is 0.038. $P < 0.05$ that there is a significant different of pulp diameter of white rat lymph in every treatment. White pulp diameter in control P_0 is 155.6 \pm 22.517. While the average in treatment and given *Nanas Bongsai* with three serial dosage P_1 , dosage P_2 , dosage P_3 is 136.933 \pm 3.349; 153.267 \pm 13.927 and 136.067 \pm 3.7 respectively. Real differences from all treatments found in positive control (Mefenammat Acid) which is different from given *Nanas Bongsai* extract with three different dosage serial 166.333 \pm 15.358. According to Affah *et al* (2014), the observed effect from Ginger (*Zingiber officinale*), is dose dependent as greater effects were seen in animals that were administered high doses. This effect may be due to the highlighted phytochemicals contained in the plant.

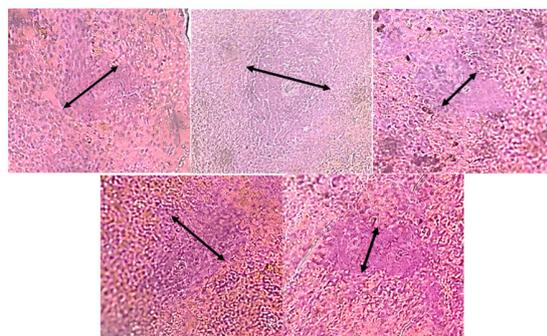


Figure 2. Histology structure lymph of *Rattus norvegicus*. Hematoxylin-Eosinstaining. 640x magnification. A: P_0 control, B: P_+ control, C: *Nanas Bongsai* Dosage 1, D: *Nanas Bongsai* Dosage 2, E: *Nanas Bongsai* Dosage 3. () white pulp

Based on result the average of white pulp treatment with given *Nanas Bongsai* extract dosage P_2 has the smallest white pulp diameter (Figure 2). Different size of white pulp diameter caused by activity enhancement of white pulp cells. This is a form of stimulation of defense system towards foreign substances that enter to the body. This circumstance can be seen from white pulp diameter and become widened indicates that immune system in lymph become increased (Abdelhafez *et al.*, 2015; Omar, 2012). In addition dif-

ferent size of white pulp in given *Nanas Bongsai* extract with three different dosage serial can caused by phytochemistry compound in *Nanas Bongsai* Extract. According to Parasuraman (2011), every active substance has pharmacology effect and potentially has toxic effect.

Microscopic Observation of Uterus

The uterus is the major female reproductive organ of most mammals. Uterus is organ for zygote implementation. The female rabbit has a bicornuate duplex uterus. Each uterus has its own cervix, and the two cervixes open into a single vagina. Many of fertility problems in mammals are of uterine origin (Abd-Elkareem, 2017). This organ consisted of the endometrium; the mucosa, the myometrium, the thick muscular layer and the perimetrium; the serosa. The endometrium was equipped with gland to supply nutrition to fertilized ovum. Endometrium and myometrium were part of tissue that plays a role in implementation to determine individual fertility that was consisted of lamina epithelialis of simple columnar type and connective tissue lamina propria containing leucocytic infiltration and endometrial glands (Brosens *et al.*, 2013; Faria *et al.*, 2015). The myometrium was formed of an inner circular (IC) and outer longitudinal (OL) smooth muscle fibers separated by tunica vascularize (layer of loose connective tissue containing blood vessels). The perimetrium was formed of a mesothelium and submesothelial loose connective tissue (Abd-Elkareem, 2017).

In this research, observation towards microscopic structure of white rat uterus after given treatment (P_0 and P_+ control) and given *Nanas Bongsai* extract with three different dosage serial. Observed result including the thickness of endometrium and myometrium. Data analysis of ensometrium and myometrium were presented in Table 2.

Table 2. Thickness average of endometrium and myometrium of white rat.

Treatment	Endometrium	Miometrium
P_0	254±57.236 ^a	104±27.622 ^a
P_+	284.667±64.933 ^a	84±36.592 ^a
P_1	206.333±33.486 ^a	97.667±6.351 ^a
P_2	215.667±33.486 ^a	60.333±14.434 ^a
P_3	197.667±60.871 ^a	73.333±40.104 ^a

Notes: P_0 : zero control P_+ : positive control, P_1 :

Nanas Bongsai Dosage 1, P_2 : *Nanas Bongsai* Dosa-ge 2, P_3 : *Nanas Bongsai* Dosage 3. Numbers followed by different alphabet at same column is significantly different at α 5%

Based on histology observation from the thickness of endometrium and myometrium that ANOVA test resulting P for 0.55. P value less than 0.05 and presumed that there is no significant changes between P_0 control (feed and drink in ad libitum) P_+ control (Mefenamat Acid) and given *Nanas Bongsai* extract with three different dosage serial. Towards the thickness of whote rat endometrium. Based on table 2 show the average of endometrium thickness at three different dosage serial did not show any significant changes of endometrium thickness. Same result with positive control (Mefenamat Acid). The average of endometrium thickness with three different dosages serial are 206.333±33.486; 215.667±33.486; 197.667±60.871 respectively. The lowest thickness of endometrium layer found in treatment of dosage P_3 . The result of this study revealed no pathological effects in the uterus of the rats. Based on Adkar *et al* (2014), the histology of uterus of mice in control group found normal whereas mice treated with of *Trapa bispinosa* Roxb. leaves with lower and higher dose (200 and 400 mg/kg body weight respectively), did not changed in the histology of uterus as compared to control group. This is in line with Amabe *et al* (2010) who reported normal uterine morphologies, and functions with the seed oil extract of *Azadirachta indica*.

Based on the table endometrium thickness of endometrium layer at positive control occurs an enhancement while treatment with given *Nanas Bongsai* extract underwent a declining along with the improvement of given dosage towards the rats. ANOVA result did not show any significant changes in every treatment. However, based on counting result of endometrium thickness at the table show that given extract of *Nanas Bongsai* at three different dosages serial affect the thickness of endometrium layer of white rat uterus. Table 2 show the average of myometrium thickness of white rats after given treatment. Based on the table there is significant difference of myometrium thickness from each treatment. ANOVA test result show that P value is 0.371. P less than 0.05 it means that there is no significant difference of white pulp diameter of white rat lymph in every treatment.

Table 2 shows that thickness in myometrium at positive control and given extract of *Nanas Bongsai* compared to normal condition.

Based on table 2 myometrium at normal control is 104 ± 27.622 while given extract of *Nanas Bongsai* at dosage P_1 , P_2 and P_3 shows a decline in myometrium thickness. However, given extract at dosage 3 (73.333 ± 40.104) is higher rather than dosage P_2 (73.333 ± 40.104). Declining in myometrium thickness at given extract of *Nanas Bongsai* with three different dosage serial can presume in normal condition showed by same notation in each treatment.

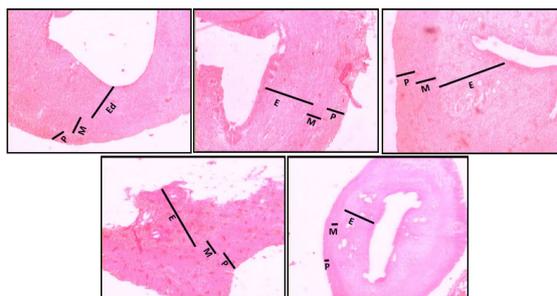


Figure 3. Histology structure of uterus *Rattus norvegicus*. SP4. Hematoxylin-Eosin staining. 640x magnification. A: control 0, B: positive control, C: *Nanas Bongsai* Dosage 1, D: *Nanas Bongsai* Dosage 2, E: *Nanas Bongsai* Dosage 3. P: Perimetrium, M: Miometrium, E: Endometrium.

Figure 3 is the description of microscopic histology of white rat uterus in all treatment including (normal control and positive control) as well as three varied dosage of *Nanas Bongsai*. Based on data analysis there is no significant effect towards endometrium layer as well as myometrium. In this figure, endometrium layer after given treatment of *Nanas Bongsai* extract. It can be seen clearly that given extract of *Nanas Bongsai* at dosage P_1 and dosage P_2 become thicker rather than dosage P_3 . While myometrium after given treatment of *Nanas Bongsai* extract show that myometrium layer in dosage P_2 show a decline compared to dosage P_1 and dosage P_3 . According Al-Qudsi & Linjawi (2012), there was a slight insignificant increase in the thickness of endometrium between the treated and the control groups and this increase was noticeable in G2 (10 mg/kg) agree with the anatomical measurements.

Plant medicinal is widely perceived by the public as being natural, healthful and free from side effects. The local community believe that Plant medicinal has no side effects or any potential risks due to their natural origins and are often considered as food supplements and not drugs (Arsad et al., 2014). All this time, pineapple was presumed able to prevent pregnancy. Consequently, this fruits is frequently used to overcome

late period of menstruation and potential as an abortivum the existence of bromelain enzyme in pineapple cause the thickness of endometrium and myometrium in uterus. In addition, *Nanas Bongsai* has high activity of bromelain enzyme and cause contraction of uterus.

From this study showed that there is no toxic effect on uterus and lymph function of *Nanas Bongsai* (*Ananas comosus* var. *microstachys* L.) at different doses, so the plant is secure for consumption by the community and will not cause toxicity effect on the limph and uterus function.

CONCLUSION

Given extract of *Nanas Bongsai* with three different dosage serial did not give significant changes can be seen from ANOVA result that have been obtained. Given *Nanas Bongsai* extract towards *R. norvegicus* can decrease white pulp diameter and damage in lymph organ compared to control treatment. Same result also obtained with given *Nanas Bongsai* extract towards *R. norvegicus* can decrease thickness endometrium layer as well as myometrium compared to control treatment. Base on this research was obtained that there is no toxic effect on uterus and lymph function of *Nanas Bongsai* (*A. comosus* var. *microstachys* L.) at different doses.

ACKNOWLEDGEMENT

Researcher utter gratitude to Public Health Office Riau Province and Ministry of Health for SP3T grants that have given to researcher. Sincere gratitude to all related parties both morale and material and this research can run well.

REFERENCES

- Abdelhafez HM, Amara TMMA, El-debsi SM. (2015). Evaluation of Role of Gliben clamide and Aphanizome non flos-aquae Extract on Lymph Node and Spleen of Diabetic Rats. *The Egyptian Journal of Hospital Medicine*. Vol 61: 700-720.
- Abd-Elkareem MD. (2017). Morphological, Histological and Immunohistochemical Study of the Rabbit Uterus during Pseudopregnancy. *Journal of Cytology & Histology*. 8(1):1-7.
- Adkar PP, Ghadge PM, Ambavade SB, Shelke TT, Bhaskar VH. (2014). Effect of hydroethanol extract of leaves of trapa bispinosa Roxb on Histology Assessment in Reproductive system of Albino Mice. *Journal of Pharmacy Research*. 8(4): 570-575.
- Affah GU, Obeten KE, Obasee PP, Isaac VN. (2014). Extract of Ginger (*Zingiber officinale*) on the

- Histology of the Spleen using Adult Male Rats. *Journal Biology, Agriculture and Healthcare*. 4(25): 259-267.
- Al-Qudsi F, Linjawi S. (2012). Histological and Hormonal Changes in Rat Endometrium under the Effect of Camphor (*Cinnamomum camphor*). *Life Science Journal*. 9(2): 348-355.
- Amabe O, Akpantah, Moses BE, Kelechi CU, Mfon AMBB, Mokutima AE, Theresa BE. (2010). Gonadal histo-morphologies and serum hormonal milieu in female rats treated with *Azadirachta indica* leaf extract. *Iranian Journal of Reproductive Medicine*. 8(4): 185-190.
- Arsad SS, Esa NM, Hamzah H. (2014). Histopathologic Changes in Liver and Kidney Tissues from Male Sprague Dawley Rats Treated with *Rhaphidophora decursiva* (Roxb.) Schott Extract. *Journal of Cytology & Histology*. S4: 001. doi: 10.4172/2157-7099.S4-001.
- Aspelund A, Robciuc MR, Karaman S, Makinen T, Alitalo K. (2016). Lymphatic System in Cardiovascular Medicine. (*Circ Res*. 2016;118:515-530. DOI: 10.1161/CIRCRESAHA.115.306544.)
- Assiam N, Iriani S, Sang KS. (2014). Pengaruh Dosis dan Lama Perlakuan Ekstrak Daun Kaliandra Merah (*Calliandra calothyrsus* Meissn.) Terhadap Struktur Histologi Ginjal Mencit (*Mus musculus* L.). *Jurnal Simbiosis* II(2): 236-246.
- Brosens, Pijinenborg, Benagiano. (2013). Defective myometrial spiral artery remodeling as a cause of major obstetrical syndromes in endometriosis and adenomyosis. *Placenta*. 34: 100-105.
- Cottencin O, Rolland B, Karila L. (2014). New Designer Drugs (synthetic cannabinoids and synthetic cathinones): review of literature. *Current Pharmaceutical Design*. 20 (25):4106-4111.
- EISAI. (1995). *Medical Herbs Index in Indonesia*. Jakarta.
- Ekor M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*. Vol 14 (117): 1-10
- El-Sherief A, Lau CT, Obuchowski NA, Mehta AC, Rice TW, Blackstone EH. (2017). Cross Disciplinary Analysis of Lymph Node Classification in Lung Cancer on CT Scanning. *CHEST*. 151 (4): 776-785.
- Faria SC, Sagebiel T, Balachandran A, Devine C, Lal C, Bhosale P. *Imaging in endometrial carcinoma*. *Indian Journal of Radiology and Imaging*. 25(2): 137-147.
- Fitmawati, Sofiyanti N, Roza RM, Isnaini, Hazimi H, Irawab YR, Winata DR, Dewi APK. (2017a). Traditional medicinal formulation: Obat pahit from Lingga Malay Ethnic in Riau Archipelago, Indonesia. *Bidiversitas*. Vol 18 (3): 1196-1200.
- Fitmawati, Roza RM, Sofiyanti N, Isnaini, Fitri FL, Paramita D, Dewi APK. (2017b). Immunomodulatory Effectiveness of Aqueous Obat Pahit Extract of Lingga Malay Ethnic on White Rats (*Ratus norvegicus*). *Biosaintifika*. Vol 9 (3): 430-436.
- Husain M, Makiyah SN. (2012). Pengaruh Pajanan Gelombang Telepon Seluler Terhadap Struktur Histologi Limpa pada Mencit (*Mus musculus*). *Jurnal Kedokteran Yarsi*. 20(3):167-173.
- Liao S, Weid PY. (2016). Lymphatic System: An Active Pathway for Immune Protection. *Semin Cell Dev Biol*. Vol 38: 83-39.
- Margaris KN, Black RA. (2012). Modelling the lymphatic system: challenges and opportunities. *Journal of The Royal Society Interface*. Vol 9: 601-612.
- Matheos C, Lintong P, Kairupan C. (2013). Gambaran Histologik Jaringan Limpa Tikus Putih (*Rattus norvegicus*) yang Diinfeksi *Eschericia coli* dan Diberi Madu. *Jurnal e-Biomedik (eBM)*. 1(2): 961-965.
- Omar NA. (2012). Effect of some Aflatoxins on a Lymphatic Organ (Spleen) of Male Albino Rats (Histopathological Study). *The Egyptian Journal of Hospital Medicine*. Vol 48: 357-367.
- Parasuraman S. (2011). Toxicology screening. *Journal of Pharmacology & Pharmacotherapeutics*. 2(2): 74-79.
- Prakash, O., Kumar, A., & Kumar, P. (2013). Screening of Analgesic and Immunomodulator activity of *Artocarpus heterophyllus* Lam. Leaves (Jackfruit) in Mice. *Journal of Pharmacognosy and Phytochemistry*, 1(6), 33-36.
- Zuhud, E.A.M. dan A. Hikmat. (1998). Eksplorasi dan kemungkinan pengembangan tumbuhan hutan sebagai bahan obat. Makalah Utama dalam Diskusi Hasil Hutan Bukan Kayu. Pusat Penelitian dan Pengembangan Hasil Hutan dan Sosial Ekonomi Kehutanan. Bogor.