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Effect Of Euchresta Horsfieldii Lesch Benn Leaf Extract On Increases Enzyme Activity Of Superoxide Dismutase And Glutathione Peroxidase In Rats with Maximum Physical Activity

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Abstract

Maximum physical activity can lead to increased metabolism and oxygen consumption so as to accelerate the formation of ROS. This study aims to determine the effect of the ethyl acetate extract of Euchresta horsfieldii lesch benn leaf on increasing the activity of SOD and GPx in blood plasma wistar rats with maximum physical activity. Of antioxidant capacity in vitro with1,1-diphenyl-2-pycrylhidrazyl where as the in vivo measurement of the enzyme activity of SOD and GPx in Wistar rats. The results showed that the ethyl acetate extract has antioxidant capacity with IC50 value of 393.95 mg/mL and total flavonoid levels of 6619.72 mg QE/100g or 6.62% QE. The extract ethyl acetate 20 mg/200g bw/day provides the best results that can increase the activity of superoxide dismutase as much as 34.93 times (171.44%) and the ethyl acetate extract of 30 mg/200g bw/day can increase levels of glutathione peroxidase were 9,76 times (41.80%). Statistical test results one way ANOVA on the mean activity of SOD and GPx after the administration of ethyl acetate extract showed the p value of 0.001 ($p<0.05$), so that the value indicates that the five treatment given to Wistar rats provide a significantly different effect.

Keywords: *Eucresta horsfieldii lesch Benn, superoxide dismutase, glutathione peroxidase, maximum Physical Activity*

INTRODUCTION

Oxidative stress is an imbalance between free radicals with antioxidants capacity in the body resulting in impaired function of the cell. The main internal factors that cause oxidative stress is oxidative phosphorylation reactions due to physical activity maximum. During physical activity will be formed in conjunction with the free radical oxidation reactions to form adenosine Triphospate phosphorylation (ATP) in the mitochondria. In such reactions needed oxygen to form water, but the amount of oxygen can be transformed into free radicals. The more strenuous physical activity it takes a lot of ATP, so that the free radicals produced as a byproduct is also more and more (Pangkahila, 2007). The body actually does have the ability to neutralize free radicals in the presence of endogenous antioxidants such as superoxide dismutase (SOD) and Gluthation peroxidase (GPx). Free radicals are formed in excess caused the imbalance amount of endogenous antioxidants and free radicals in the body that cause oxidative stress (Pangkahila, 2007; Sen *et al*, 2010).

To increase the activity of endogenous antioxidants in preventing oxidative stress, and it would require extra antioxidants from outside the body (exogenous antioxidants), one of which is a flavonoid compound. Flavonoids potential as an antioxidant because it can donate H^+ ions on free radical compounds (Kandaswani and Middleton, 1997). It is supported by several studies, namely the n-butanol fraction Dutch eggplant can increase the activity of SOD and reduce levels of MDA. N-butanol fraction were allegedly contains flavonoids class of flavones, flavonols and isoflavones may contribute as natural antioxidants (Widayanti, 2015). Also note that the administration of red pomegranate juice may also increase blood levels of GPx in mice with maximal physical activity (Sugianto, 2011).

According to Intan Sari *et al* (2015), isolates the active n-hexane extract of Euchresta horsfieldii lesch benn leaf at a concentration of 8000 ppm has a free radical activity with a percentage reduction of 94,67%. Also note the n-hexane extract of Euchresta horsfieldii lesch benn leaf has antioxidant capacity of 126.94 ppm GAEAC (Garlic Acid Equivalent Antioxidant Capacity) (Tirta and Ardaka, 2010). In granting seed extract Euchresta horsfieldii lesch benn also can improve the rate of cell damage-β pancreas with an indication of decreasing levels of blood glucose, AGEs (Advanced Glycation End Products), MDA (malondialdehyde), and 8-OHdG (8-hydroxy-2-dioksiguanosin) in wistar rats hyperglycemia (Gunawan *et al*, 2015).

Based on these studies, it can be stated Euchresta horsfieldii lesch benn leaf is one source of antioxidants, but no studies have reported whether the ethyl acetate extract of the Euchresta horsfieldii Lesch benn leaf can increase the activity of superoxide dismutase (SOD) and the of glutathione peroxidase (GPx) levels in rats wistar oxidative stress after maximal physical activity. With this premise, the ethyl acetate extract of the Euchresta horsfieldii lesch benn leaf on wistar rats after experiencing maximum physical activity they need to be investigated further.

MATERIAL AND METHODS

Preparation of plant extract

Euchresta horsfieldii lesch benn leaf obtained from Bukit Tapak, Bedugul, Tabanan. A total of 2.5 kg of Euchresta horsfieldii Lesch benn leaf washed and cut into small pieces, then later wind dried for ± 5 days. The dried samples are dry blended in order to obtain the material in powder form. The powder was macerated with n-hexane for 48 hours and diremaserasi again five times, in order to obtain a thick extract n-hexane. The extract is then filtered and the filtrate is then concentrated in a rotary evapotarator at a temperature of 45ºC to extract the thick n-hexane. The dregs of the results of the re-macerated macerating with ethyl acetate to obtain too thick ethyl acetate extract. Having obtained a viscous extract, phytochemical test done to determine the content of secondary metabolites contained in ethyl acetate extract thick.

Experimental Design

This research is a descriptive exploratory study to determine the ethyl acetate extract of the Euchresta horsfieldii lesch benn leaf which acts as an antioxidant while experimental methods include measuring levels of MDA, activity of SOD and GPx with the draft *pre and posttest control group design* (Petrie Sabin, 2003). The study used 25 rats Wistar divided into five groups: control negative (Po), the control group was positive by vitamin C (K), the treatment group the ethyl acetate extract with various doses (P_1 = 10 mg/200g bw/day, P_2 = 20 mg/200g bw/day, and P_3 = 30 mg/200g bw/day)

Experimental Animal

The ethyl acetate extract of Euchresta horsfieldii lesch benn leaf continued in test animals that Wistar rats with various concentrations and used as a comparison negative control (distilled water) and positive control (vitamin C). Before the treated extract, adapted test animals for seven days and do perenangan for 5 days ± 60 minutes per day and then have blood drawn. After the randomization and wistar rats were divided into five groups: negative control (P0), a positive control (K), and the treatment group ethyl acetate extract of the Euchresta horsfieldii Lesch benn leaf respectively P_1 = 10 mg/200g bw/day, P_2 = 20 mg/200g bw/day, and P_3 = 30 mg/200g bw/day for 21 days. After the mice have blood drawn. Measurement of MDA done only when the pre-test only to find out that the rats are already experiencing oxidative stress. Measurement of MDA, SOD and GPx using spectrophotometric methods (Sun *et al*, 1988; Mudasir *et al*, 2011).

Measurement of Malondialdehyde (MDA) Levels

Rat blood sample preparation performed following the method of Singh *et al* (2002). 0.5 ml of rat blood plus 2.0 ml of cold HCl (0.25 N) containing 15% TCA; 0.38% BHT. The mixture was heated at 80°C for one hour. Once cool, the mixture was centrifuged for 10 minutes. Serum absorbance was measured at λ 532 nm. As a standard solution used *MDA Assay Kit*.

Measurement of SOD Activity in Rats

A total of 0.06 ml plasma reacted with a mixture of 2.70 ml of sodium carbonate buffer containing 0.1 mM EDTA (pH 10), 0.06 mL of 10 mM xanthine, 0.03 ml of 0.5% bovine serum albumin, 0,03 ml of 2.5 mM NBT. Furthermore, the addition of xanthine oxidase (0.04 units). The resulting absorbance after 30 minutes was measured at a wavelength of 560 nm. As a control used PBS solution containing 11.5g/L KCl. SOD activity (%) was calculated using the equation: (1- (A/B)) x 100% (Kotan *et al*, 2011).

Measurement of GPx Activity in Rats

A total of 200 mL rat plasma was added to 200 mL of 0.1 M phosphate buffer pH 7.0 containing 0.1 mM EDTA reduced glutathione (GSH) and 200 mL of 10 mM glutathione reductase enzyme. Furthermore, incubated for 10 min at 37°C, was added 200 mL of 1.5 mM NADPH and incubated again for three minutes at the same temperature, followed by addition of 200 mL of 1.5 mM H_2O_2. Absorbance was measured with a spectrophotometer at a wavelength of 340 nm (Kotan *et al*, 2011; Wrasiati, 2011).

Data Analysis

All the data obtained are described, then performed statistical analysis as follows:

1. Analysis of normality using the Shapiro-Wilk test with significance level $\alpha = 0.05$
2. Analysis of homogeneity of variance using Levene test test
3. The comparative tests using one way Anova to know the difference between group and followed by Post Hoc test Least Significant Differences test
4. Furthermore, the different Duncan test, whether each group before and after treatment showed different results with $p<0.05$

RESULT AND DISCUSSION

Extraction of Plants

2.5 Kg of Euchresta horsfieldii lesch benn leaf obtained material in powder form to 580 g. The powder was macerated with solvent n-hexane to obtain the thick extract n-hexane as much as 5.18 g. The dregs of the results of the re-macerated macerating with ethyl acetate to obtain too thick ethyl acetate extract as much as 13.16 g. Condensed ethyl acetate extract obtained positive results for the compound class of flavonoids, alkaloids, steroids, and phenol.

Enzyme activity of SOD and GPx

Before the ethyl acetate extract of Euchresta horsfieldii lesch benn leaf on wistar rats, first measured of MDA levels to determine the mice suffer from oxidative stress, further examination of the SOD and GPx activity. MDA average obtained at 9.32 mol/L. MDA levels were higher in wistar rats occurs due to excessive physical activity treatments that cause oxidative stress in wistar rats. MDA levels normal by spectrophotometric method was 1.04 ± 0.43 mol/L (Asni, 2009).

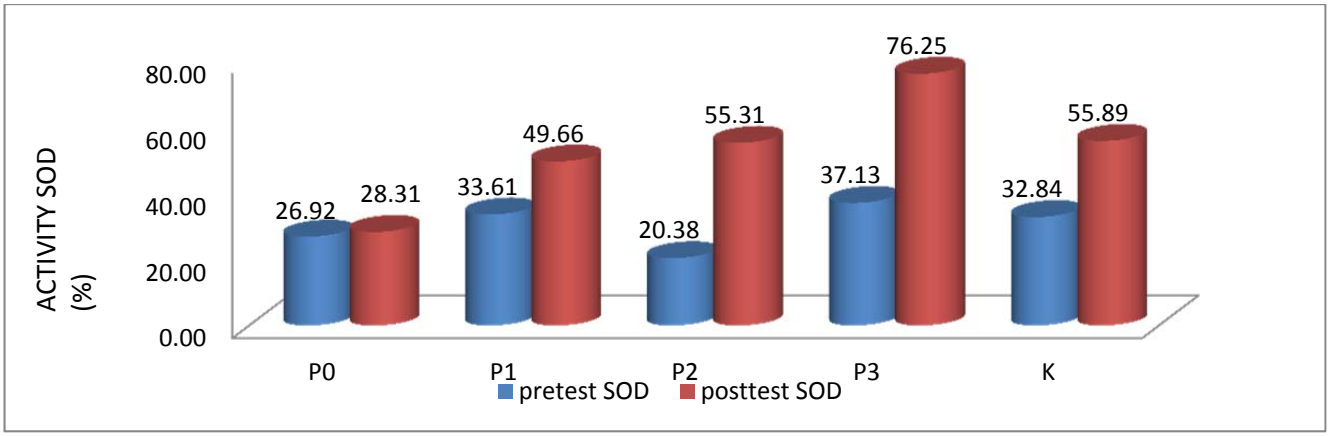

Figure 1. SOD activity pretest and posttest administration of the ethyl acetate extract Euchresta horsfieldii lesch benn

Results of statistical analysis of the superoxide dismutase activity in each treatment group after the administration of ethyl acetate extract Euchresta horsfieldii lesch benn indicates that the test for normality using the Shapiro-Wilk test produces normal distributed data because it has a value of p>0.05. Results of homogeneity test using test Levene's test shows that the data are homogeneous with p value of 0.115 (p>0.05). One way ANOVA test results to mean SOD activity showed the p value of 0.001 (p<0.05), so that the value indicates that the five treatment given to wistar rats provide a significantly different effect. Provision of ethyl acetate extract of leaves Euchresta horsfieldii Lesch benn with a concentration of 30 mg/200g bw/day for 21 days after the mice given the treatment physical activity in excess has been able to increase the activity of SOD, in which the active compound in the ethyl acetate extract of the Euchresta horsfieldii lesch benn leaf able to react with free radicals in the body. The increased of SOD activity after administration of the ethyl acetate extract is also suspected due to the induction of genes responsible for the synthesis of antioxidant enzymes through Nrf_2 translocation to the nucleus to increase the expression of genes encoding antioxidant. Furthermore Nrf_2 will bind the ARE (Antioxidant Response Element) in the target genes and induces gene antioxidants (Dawn *et al*, 2000).

Results of further tests using LSD Post Hoc test SOD activity showed that the average difference is highest in the group of rats treated P_0 and P_3. Overall, the treatment given to the rats of the wistar different effects significantly. Further statistical analysis by Duncan test $\alpha = 0.05$ indicates that the treatment between groups within the subset is not significantly different, but between there is significant subset. As for the activity of superoxide dismutase of treatment pretest and posttest administration of the ethyl acetate extract Euchresta horsfieldii lesch benn can be seen in Figure 1.

Figure 1 shows the increase of SOD activity pretest and posttest administration of the ethyl acetate extract. After treatment provision of the ethyl acetate extract Euchresta horsfieldii lesch benn leaf against wistar rats at dose of P_1 = 10 mg/200g bw/day, P_2 = 20 mg/200g bw/day, P_3 = 30 mg/200g bw/day and vitamin C (ascorbic acid) as a positive control (K) with a dose of 10 mg/200g bw/day an increase in SOD activity. The increase in activity can be seen in Table 1.

Table 1 The Increase of SOD Activity

Treatment	Before giving extract	After giving extract	The increase of SOD activity	% Increase of SOD activity
P_0	26.92	28.31	1.39	5.18
P_1	33.61	49.66	16.05	47.76
P_2	20.38	55.31	34.93	171.44
P_3	37.13	76.25	39.11	105.34
K	32.84	55.89	23.05	70.17

Based on the data in Table 1 shows that the ethyl acetate extract Euchresta horsfieldii lesch benn potentially increasing SOD activity in wistar rats given maximum physical activity. The highest percentage increase in SOD activity are those of P_2 is equal to 171.44%. This shows of the ethyl esetat extract Euchresta horsfieldii lesch benn leaf a dose of 20 mg/200g bw/day showed a very high increase of the SOD activity after maximal treatment physical activity. The increase was due of Euchresta horsfieldii lesch benn extract leaf with a dose of 20 mg/200g bw/day already showed the SOD activity was within the normal range or there is a state of homeostasis. P_3 group (treatment of ethyl acetate extract of Euchresta horsfieldii lesch benn a dose 30 mg/200g bw/day) provides enhanced of SOD activity by 105.34%. P_3 group gives rise antioxidant activity was lower than P_2 possibility for SOD activity already at the level that is sufficient, thereby granting the ethyl acetate extract Euchresta horsfieldii Lesch benn be not very influential. While the ethyl acetate extract of leaves Euchresta horsfieldii lesch benn a dose of 10 mg/200g bw/day (group P_1) shows the percentage increase in SOD activity by 47.76% compared to the positive control, namely vitamin C by administering 10 mg/200g bw/day (group K) obtained SOD percentage increase of 70.17%. P_0 group (treatment administration of distilled water) provide improved SOD activity of 5.18%

Results of statistical analysis of the glutathione peroxidase (GPx) levels after administration of the ethyl acetate extract Euchresta horsfieldii Lesch benn in each

treatment group showed that the test for normality using the Shapiro-Wilk test produces normal distributed data because it has a value of p>0.05. Results of homogeneity test using test Levene's test shows that the data are homogeneous with p value of 0.115 (p>0.05). One way ANOVA test results to the average of GPx levels after the ethyl acetate extract showed the p value of 0.001 (p<0.05), so that the value indicates that the five treatment given to wistar rats provide a significantly different effect. The highest of GPx levels is owned by K treatment group (positive control of vitamin C a dose 10 mg/200g bw/day) in the amount of 35.56 U/mL. Vitamin C is a water soluble vitamin that is only able to eliminate free radicals in a liquid medium. Vitamin C has the ability to suppress free radicals that attack lipids. As a free radical scavenger, vitamin can directly react with superoxide and hydroxyl anion, as well as a variety of lipid hydroperoxide. Vitamin C also acts as an antioxidant secondary to maintaining the reduced glutathione as an important antioxidant. With these capabilities allow a synergistic relationship with other antioxidants (antioxidant network), so as to maintain and improve the ability as an antioxidant (Bier *et al*, 2004).

The potential of the ethyl acetate extract of the Euchresta horsfieldii lesch benn leaf can also be seen in the of GPx levels in $P_1<P_2<P_3$. This suggests the addition of ethyl acetate extract of Euchresta horsfieldii lesch benn potentially increasing of GPx levels in wistar rats given maximum physical activity. The content of flavonoids in leaf extracts Euchresta horsfieldii lesch benn give effect to the possibility of increased of GPx levels in the wistar rat. Further statistical analysis by Duncan test $\alpha = 0.05$ indicates that the treatment between groups within the subset is not significantly different, but between there is significant subset. The comparison of the levels of glutathione peroxidase of treatment before and after the administration of ethyl acetate extract Euchresta horsfieldii lesch benn can be seen in Figure 2.

Figure 2 shows that not all treatments there was an increase of GPx levels pretest and posttest administration of the ethyl acetate extract Euchresta horsfieldii lesch benn. Treatment award ethyl acetate extract on wistar rats with a concentration of 10 mg/200g bw/day (P_1) did not show increased of GPx levels in wistar rats, whereas the administration of ethyl acetate extracts of Euchresta horsfieldii lesch benn a dose of 20 mg/200g bw/day, 30 mg/200g bw/day (P_2, P_3) and vitamin C as a positive control (K) with a concentration of 10 mg/200g bw/day occurs the rising of GPx levels. Increased of GPx levels can be seen in Table 2.

Table 2. The Increase in GPx Activity

Treatment	Before giving extract	After giving extract	The increased of GPx levels	% Increased of GPx levels
P_0	22.79	20.69	-2.10	-9.21
P_1	23.43	22.40	-1.03	-4.40
P_2	22.86	29.81	6.94	30.36
P_3	23.36	33.12	9.76	41.80
K	23.70	35.56	11.86	50.03

Based on the data in Table 2 shows that the ethyl acetate extract Euchresta horsfieldii lesch benn in wistar rats has the potential to increase the of GPx levels at a dose of 20 mg/200g bw/day. While the ethyl acetate extract 10 mg/200g bw/day did not increase of GPx levels wistar rats, resulting in decreased of GPx levels amounted to 4.40%. The higher of peroxidase gluthathion meant as an endogenous antioxidant activity is increasing. Conversely, when a decline in mean levels decreased activity (Sugianto, 2011). Plasma GPx levels associated with plasma selenium levels. Decreased levels of GPx also showed a decline in the levels of Selenium. It is associated with the relationship between decreased activity of GPx with arterial thrombosis, the clinical picture of ischemic stroke, and coronary artery disease proved that this enzyme is important for maintaining vascular homeostasis (Bierl, 2004). In addition to the decreased levels of GPx suspected occurrence due to the extract a dose of 10 mg/200g bw/day has shown the optimum concentration of the extract in the body. The ethyl acetate extract Euchresta horsfieldii lesch benn contain flavonoid compound binds to SOD in advance helps reduce free radicals superoxide ($O_2\cdot$) and help the work of the enzyme catalase. So that when reacted with GPx, the extract is already in a state of saturation. This leads to decreased of GPx levels. While the ethyl acetate extract Euchresta horsfieldii lesch benn a dose of 20 mg/200g bw/day had increased of GPx levels because the concentration of the extract has been able to increase GPx antioxidant gene transcription mediated by Nrf_2.

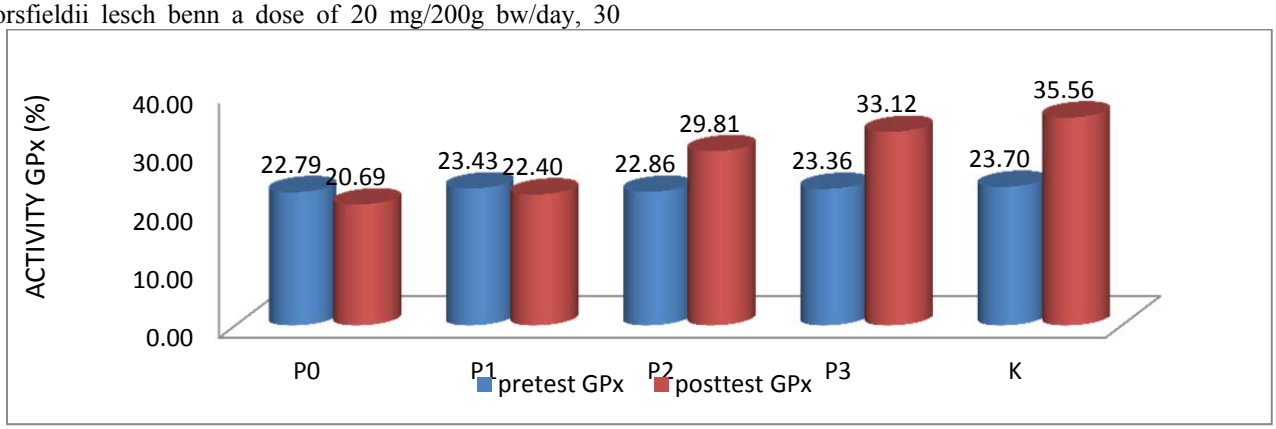

Figure 2. GPx levels pretest and posttest administration of the ethyl acetate extract Euchresta horsfieldii lesch benn

CONCLUSION

Based on the results of research and discussion, it can be concluded that the ethyl acetate extract of Euchresta horsfieldii lesch benn leaf a dose of 20 mg/200g bw/day has been able to increase the activity of superoxide dismutase by 171.44%, while the ethyl acetate extract of the Euchresta horsfieldii lesch benn leaf as much a dose of 10 mg/200g bw/day is only able to provide the increased activity of superoxide dismutase of 47.76%, when compared to controls vitamin C (ascorbic acid) 10 mg/200g bw/day which provides enhanced superoxide dismutase activity of 70.17%. Ethyl acetate extracts of Euchresta horsfieldii lesch benn leaf as much a dose of 30 mg/200g bw/day has been able to increase of the GPx levels by 41.80% compared with the controls vitamin C (ascorbic acid) a dose 10 mg/200g bw/day which provides increased of GPx levels 50.03%, while the ethyl acetate extract of the Euchresta horsfieldii lesch benn as much a dose of 10 mg/200g bw/day decreased of GPx levels amounted to 4.40%.

REFERENCES

1. Asni, E. 2009. Toward Sustainable Hypoxia Influence Levels of Malondialdehyde, Reduced Glutathione and Catalase Activity Rat Kidney. *Indonesia Scientific Journal*. Jakarta.
2. Bierl, C., Voetsch, B., Jin, R.C., Handy, D.E., Loscalzo, J. 2004. Determinants of Human Plasma Glutathione Peroxidase (GPx-3) Expression. *The Journal Of Biological Chemistry*, 279:26839-26845
3. Dawn, B.M.; Allan, D.M., and Smith, C.M. 2000. Basic Medical Biochemistry: a clinical approach. p. 321-523. Jakarta: EGC.
4. Gunawan I W.G., Bawa I G.A.G, and Sutirta Yasa W.P. 2015. Effects of Pranajiwa Seed Extract Repair Damaged Pancreatic B-Cells through Decrease of Blood Glucose Levels, Advanced Glycation end-Products and Profile Histopathology in Hyperglycemic Rats. *J.Biol.Chem.Research*. 32(1): 299-312.
5. Kandaswani, C. dan Middleton, E. 1997. Flavonoids as Antioxidant. In: Shahidi, F., editor. Natural *Antioxidant Chemistry Health Effects and Applications.* Champaign Illions: AOAC Press.
6. Kotan, E.,Alpsoy, L., Anar, M., Aslan, A and Agar, G. 2011. Protective Role of Methanol Extract of Cetraria Islandica Against Oxidative Stress and Genotoxic Effects of AFB in Human Lymphocytes in Nitro, Toxicology and Industrial Health, 27(7): 599-605
7. Mudasir, A. Azis, A.Q. Punagi. 2011. "Plasma MDA levels Analysis of Patients with nasal polyps Inflammatory Cells Based Dominance In histopathology examination "(thesis). Makasar: University of Hasanudin.
8. Pangkahila, W. 2007. Slowing Aging, Quality of Life. Anti-Aging Medicine. Molds to-1. Jakarta. Compass Book Publishers. 9: 106-108
9. Petrie, A and Sabin, C. 2003. Medical Statistic at a Glance, Massachusetts: Blackwell Science Inc. 26-7
10. Sari, A. I., Gunawan, I W.G., dan Putra, K.G.D. 2015. Compound Antioxidant Capacity Group Triterpenoid On Leaves Pranajiwa (Euchresta horsfieldii Lesch Benn). *Jurnal Kimia 9* (1) : 61-66.
11. Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y.S.R And Biplab, D. 2010. Free Radicals, Antioxidants, Diseases and Phytomedicines: Current Status and Future Prospect, International Journal of Pharmaceutical Sciences Rivew and Research, 3(1): 021
12. Sugianto, N.L. 2011. "Giving Red Delima Juice (Punica granatum) can Increase Blood Glutathione peroxidase Levels in Mice (Mus musculus) with a maximum of Physical Activity", Denpasar: University of Udayana
13. Sun, Y., Oberley, L. W., dan Li, Y. 1988. A Simple Methode for Clinical Assay of Superoxide Dismutase. *Clin.Chem.* 34 (3): 497-500
14. Tirta, I.G., Ardaka, I.M., dan Darma, I.D.P. 2010. Phenology studies Pronojiwo And Chemical Compounds (Euchresta horsfieldii Lesch Benn). *Bul. Littro*. 21(1): 28 – 36
15. Widayanti, N.P. 2015. "Identification of Active Fraction of Skin Extract N-Butanol Terong Belanda (Solanum betaceum Cav.) And Test of Its activity against superoxide dismutase In Rats Under Stress Conditions" (*thesis*). Denpasar: University of Udayana
16. Wrasiati, L.P. 2011. Characteristics and Toxicity Kamboja Flower Extract Powder Crude (Plumeria alba) and their Role in Improving Enzymatic Antioxidant Activity in Sparague Dawley Rats. Denpasar, University of Udayana
17. Wresdiyati, T., Astawan, M., Fitriani, D., Adnyane, I.K.M., Novelina, S., dan Aryani, S. 2013. Effect of α-Tocopherol On The Profile Superoxide Dismutase and Malondialdehida in Rat Liver Under Stress Conditions, *Jurnal Veteriner*, 2:2-9
18. Zelko, I.N., Mariani, T.J., And Folz, R.J. 2002. Superoxide Dismutase Multigene Family: A Comparison of The CuZn-SOD (SOD_1), Mn-SOD (SOD_2), and EC-SOD (SOD_3) Gene Structure, Evolution and Expression, *Free Radical Biology & Medicine*, 33(3): 337-49