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Stimulant Activity of White Pepper (*Piperis albi* Fructus) Ethanol Extract In Vivo

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ABSTRACT

Caffeine is a stimulant substances that can stimulate the central nervous system (CNS) increasing concentration, and reducing drowsiness. The use of caffeine generally comes from coffee plants which can increase gastrin secretion. Long-term use of caffeine can cause side effects such as feelings of anxiety, irritability, insomnia and faster heart rate. An alternative stimulant that has limited side effects is needed. White pepper (Piperis albi fructus) is potential as stimulant. The extract of White pepper contained piperine compound. Piperine can potentially be a stimulant because it can stimulate the formation of energy in the body. The purpose of this study was to determine the in vivo stimulant activity of ethanol extract White pepper in male white mice. The method used in this research is Natatory exhaustion. 25 test animals were divided into 5 groups. The doses used are white pepper extract 0.32; 0.65 and 1.3 mg/20 g BW, caffeine 0.26 mg/20 g BW and 0.5% CMC-Na. Data analysis with SPSS 16 with the Kruskal-Wallis test and the Mann-Withney test. The results of the stimulant activity test of white pepper ethanol extract at a dose of 0.32; 0.65 and 1.3 mg/20 g BW for male white mice with the DDY strain with an average % increase in stamina of 63.92; 120.81 and 179.31%,mean while in the positive control of caffeine by 232.55% and the negative control of CMC-Na by 21.10%. White pepper ethanol extract dose of 1.3 mg/20 g BW has stimulant activity which is not significantly different from caffeine positive control.

INTRODUCTION

Energy is needed by human to carry out physical activities. Energy needs increase with heavy physical activity, so it requires a lot of energy to eliminate the feeling of fatigue that occurs (Setyowati et al., 2014). Physical activity can occur due to muscle concentrations. Energy in the form of adenosine triphosphate (ATP) is required for muscle contraction. adenosine triphosphate is then hydrolyzed to adenosine diphosphate (ADP) and used for muscle contraction. The limited amount of adenosine triphosphate in the body causes short muscle contractions and causes fatigue (Herwana et al., 2005).

Fatigue is a condition where the body experiences a lack of energy. To eliminate fatigue a person needs to consume stimulants (Mustopo, 2011; Setyowati et al., 2014). Stimulants can cause a person to increase enthusiasm, reduce fatigue and increase alertness. Stimulants work by speeding up the central nervous system (CNS) as the controller of all body activities and the center for regulating information Central nervous system stimulation by stimulants can increase physical and mental abilities, concentration, so that it can make a person more prepared and minimize fatigue (Katzung, 2014; Tary & Sujono, 2018; Wade & Travis, 2008).

The substance that is widely used by people as a stimulant is caffeine which comes from

coffee, which can reduce fatigue and drowsiness. However, long-term use of caffeine can cause side effects, feelings of anxiety, restlessness, irritability, insomnia and a faster heart rate. In the gastrointestinal system, caffeine can increase gastrin secretion, increasing gastric acid secretion. High stomach acid causes inflammation and erosion of gastric mucosa and cause dyspepsia so that not everyone can consume coffe. Therefore, alternative stimulants are needed that have minimal side effects (Fithriyana, 2018; Rahmi et al., 2020).

White pepper (*Piper albi fructus*) is a member of the Piperaceae family which has potential as a stimulant. Based on previous research, White pepper has antihypertensive, antiasthmatic, antimicrobial, antioxidant, anticancer, anti-inflamatory, hepato-protective, antidiarrheal, antidepressant, immunomodulatory, anticonvulsant and analgesic properties (Damanhouri, 2014).

White pepper contains essential oils (1.2%), fat (6.5-7.5%) and piperine alkaloids (5.3-9.2%) (Boangmanalu & Zahrotun, 2018). The piperine compound in black pepper has anti-inflammatory activity (Levita et al., 2019), antioxidant, antibacterial, anti-microbial, antitumor, cytotoxicity. Piperine stimulates a dose-dependent increase in the secretion of gastric acid and interruption of gastrointestinal motility. The oral administration of piperine activates the liver, pancreas and digestive enzymes in the small intestinal mucosa. The addition of piperine in food materials as food flavours may increase the protease, lipase and pancreatic amylase activities (Ashokkumar et al., 2021).

The piperine compound is reported to have four isomers namely piperine, isopiperine, chavicin and isochavicin (Damanhouri, 2014). The piperine compound in black pepper has the potential as a stimulant and the chavicin compound has the potential to stimulate enthusiasm (Gorgani et al., 2017). There are differences in piperine levels in black pepper and white pepper. The 96% ethanol extract of black pepper and white pepper contain piperine levels of 36.97 ± 11.95% and 38.72 ± 8.28% respectively (Hikmawanti et al., 2016).

Based on the description above, there is no information regarding the stimulant activity of white pepper ethanol extract, so research was carried out on the potential stimulant activity of white pepper extract which can reduce fatigue due to work activities

METHODS

Materials

White pepper was collected from Cigeulis Pandeglang, 96% ethanol, HCl, Mg Powder, amyl alcohol, ammonia, chloroform, H2SO4, Mayer reagent, Wagner reagent, FeCl3, anhydrous acetic acid, 25 male white mice of the Deutschland Denken Yoken (DDY) strain, Caffein, CMC-Na, distilled water.

Plant Determination

Twenty (20) kg of white pepper was collected from Cigeulis Pandeglang, Banten. Plants were determined at the Herbarium Bogoriense, Directorate of Scientific Collection Management at BRIN Cibinong.

Sample Preparation and Extraction

White pepper is prepared by soaking in water, so that the skin peels off. Then the samples were cleaned, dried and ground. The powder of white pepper was sieved using mesh No. 60. White pepper was macerated using 96% ethanol solvent in a ratio of 1:2 w/v for 3 x 24 hours. The macerate was concentrated using a vacuum rotary evaporator until a concentrated extract was obtained.

Phytochemical Screening

Flavonoid identification

As much as 1 g of simplicia white pepper was added to 10 mL of hot distilled water, then heated for 10 minutes and filtered. 2 mL of filtrate was added with 1 mL of concentrated HCl, and 0.1 g of Mg Powder and 2 mL of amyl alcohol. The color change in the amyl alcohol layer to yellow, red or orange indicates that the white pepper contains flavonoid compounds (Hasibuan & Edrianto, 2021).

Alkaloid Identification

As much as 1 g of simplicia was added with 10 mL chloroform and a few drops of ammonia. The chloroform fraction was separated then concentrated H2SO4 was added. 0.5 mL of the fraction was taken and 2 drops were added to each Mayer and Wagner reagent tube. The presence of white precipitate in Mayer's reagent and a brown precipitate in Wagner's reagent

indicates that white pepper contains alkaloid compound (Syafitri et al., 2014).

Saponin Identification

As much as 1 g simplicia white pepper was diluted with 10 mL of hot distilled water, then shaken vigorously for 15 minutes. If the foam dose not dissapear with the addition of 1 drop of HCl, this indicates the presence of saponin (Syhadat & Siregar, 2020).

Tannin Identification

As much as 1 g of simplicia white pepper was dissolved in distilled water, filtered. 2 mL of filtrate was added with 2 drops of 1% FeCl3 reagent. Positive results were indicated by green or blue-black color change (Hasibuan & Edrianto, 2021).

Terpenoid and Steroid Identification

As much as 1 g of simplicia white pepper was dissolved in chloroform, then filtered. The filtrate was added with 1 mL of anhydrous acetic acid and 2 mL of concentrated H2SO4 (Lieberman Bucard test). Terpenoid positive if a red or purple color forms and steroid positive if a green or blue color forms (Rahimah et al., 2019).

In Vivo Stimulant Activity Test

Review the Ethics of Experimental Animals

The ethical review was carried out the Medical and Health Research Ethics Commission, Muhammadiyah University, Prof. Dr. Hamka (KEPKK-UHAMKA). Ethical reviews were carried out to test the feasibility of research and to find out the reason for using test animals by considering the suffering that will be experienced by the test animals and the benefits that will be obtained for human (Ridwan, 2013).

Preparation of Experimental Animals

The sample size for experimental animals used the Federer formula, namely

$(n-1)(t-1) \geqslant 15$ .....(1)

n = repetition

t = treatment (number of treatments was 5)

The number of experimental animals that will be used is 25 male white mice of the Deutschland Denken Yoken (DDY) strain (Uthia et al., 2017). Before the research, mice were acclimatized for 7 days for environmental adjustment, supervision and uniformity of food (Isnenia, 2017).

Stimulant Test

Experimental animals were fasted for 3-4 hours, aimed at emptying the stomach to speed up drug absorption. The mice were then swam in an aquarium filled with water. Then the mouse's tired time was recorded using a stopwatch. Mice were tired when they lower their heads or experience struggling phase until they felt below the water surface for 7 seconds. Next, the mice were rested for 15 minutes. After that, the mice were treated according (**Table 1**). After 30 minutes of diving the sample, the mice swam back into the aquarium and the mice's fatigue time was recorded. The % increase in mice stamina was calculated (Sidrotullah et al., 2019).

$$\%\ \boldsymbol{increase\ in\ stamina} = \frac{t2-t1}{t1} \quad \text{.....(2)}$$

t1 = fatigue time before being given the test solution

t2 = fatigue time after being given the test solution

RESULT AND DISCUSION

Table 1. Stimulant test treatment of ethanol extract of P. nigrum

Treatment	Sample	Dose (mg/20 g BW)
1	White papper extract	0.32
2	White papper extract	0.65
3	White papper extract	1.3
Control (+)	Caffein	0.26
Control (-)	CMC-Na 0.5%	-

Determination results

The determination results showed that the plant specimen was indeed the *Piperis albi fructus* plant from Piperaceae family. Determination is the stage of determining the name of a specific type of plant. Carried out to determine the correctness of sample to be used (Soemarie et al., 2017).

Table 2. Identification results of P. nigrum fruit simplicia phytochemical screening

Reagent	Observation Results	Description
HCl + Mg powder + amyl alcohol	Red color	Positive flavonoid
Wagner	Brown precipitate	Positive alkaloid
Mayer	White precipitate	Positive alkaloid
Hot distilled water + HCl 2 N	Foam that didn't disappear	Positive saponin
$FeCl_3$ 1%	Blackish blue color	Positive tannin
Liebermann-Burchard	No green or blue color formed	Negative steroids
Liebermann-Burchard	Purple color	Positive triterpenoids

Sample Preparation and Extraction Results

White pepper originating from Cigeulis, Pandeglang, Banten. White pepper fruit was made into simplicia, then the samples were blended into powder and sieved using a mesh sieve No. 60. Refining aims to reduce the size of simplicia particles, thereby maximizing the extraction process by expanding the surface contact between the filter liquid and the active compounds contained in white pepper (Ahwan, 2018). White pepper simplicia powder was extracted using the maceration method with 96% ethanol solvent. Maceration aims to extract active substances that are not resistant to heating. The reason for choosing the 96% ethanol solvent was that the extraction of piperine compounds can be done with polar solvents (Wendersteyt et al., 2021).

The results of making 1000 g of white pepper extract from simplicia with 6000 mL of solvent resulted in a thick extract of 90.747 g with a yield percentage of 9.075%.

Phytochemical Screening

The results of the phytochemical screening identification carried out on the white pepper simplicia to determine the class of secondary metabolite compounds contained in white pepper. The result showed that it positively contained flavonoids, alkaloids, saponins, tannins and triterpenoids which can be seen in **Table 2.**

Results of In vivo Stimulant Activity

The results of the ethical review showed that the in vivo research protocol could be approved for implementation and passed ethical review (Ethical approval number: 03/22.08/02025).

Acclimatization Results

The male white mice of the DDY strain had an average weight before acclimatization (20.3 g) and after acclimatization for 7 days the average weight increased (28.1 g) **(Table** 3**).**

Table 3. Body weight of acclimatized mice

Body weight of mice (g) in various group				
T1	**T2**	**T3**	**T4**	**T5**
28	29	29	28	26
26	30	27	28	27
30	28	30	28	27
27	28	30	26	29
29	27	28	29	28
Average weight of mice: 28.1 g Coefficient Varians (CV): 4.46 % Terms of (CV): <15%				

The experinmental animals used in this research were male mice. Before testing, 30 mice were grouped into 6 groups, where each group consisted of 5 mice. Mice that have just arrived are weighed first to determine the initial Coefficient of Variance (CV). The initial CV results obtained were 5.77% eith an average body weight of 20.3 g. Then the animals were acclimatized for 7 days. Acclimatization aims to enable the experimental animals to adapt to the new environment and to obtain a homogeneous mouse weight. The homogeny of experimental animals can be determined by calculating the CV with a tolerance standard of <15%. The CV aims to observe data variations or the distribution of data. If the CV is smaller, the more diverse (homogeneous) the data (Efia, 2019). After acclimatization, the weight of the mice incerased with a CV value 4.46% (fulfilling the requirements) with an average body weight of 28.1 g.

Results of Stimulant Activity

In this test, mice will experience two phases. In the first phase the mice will swim their head

and both legs up (struggling phase) and after that the mice will experience a phase where the body position is no longer horizontal and the mice's four legs do not move in the water (floating phase). The floating phase ends when the mouse begins to sink and the mice is immediately lifted.

The results of the histogram data can be seen in

Table 4 showing that all treatment doses have a stimulant effect. The dose of white pepper extract at a dose of 1.3 mg/20 g BW provided the highest stimulant effect with a % increase of 179.31%. The results of caffeine control showed a % increase of 232.55%. White pepper extract doses of 0.32 and 0.65 mg/20 gBW showed a % increase in stamina of 63.92 and 120.81 respectively.

Based on data analysis using the Kruskal-Wallis test, results were obtained that were significantly different between treatments at all doses before administration of the extract with an Asymp.Sig value obtained of 0.000 (<0.05). The Mann-Whitney further test was carried out to see the differences between the treatment group and the control group. The results showed that at a dose 1.3 mg/20 g BW, white pepper extract had no significant difference with positive control caffeine with an Asmp.Sig value of 0.347 (>0.05).

The piperine compound found in black papper is an alkaloid which is efficacious for increasing stamina (Sumarny et al., 2013). The mechanism of action of alkaloid compounds as stimulants is by inhibiting the phospho-diesterase enzyme. Inhibition of the pospho-diesterase enzyme can increase the synthesis of 3'5'-adenosine monophosphate (cyclic AMP) (Febrianasari et al., 2016). An additional source of energy for the body is glucose 6 phosphate which is formed through cyclic AMP activating the phosphorylakinase enzyme, and converting glycogen in the body into glucose 1 phosphate, then with the enzyme glucophosphomutase, glucose 1 phosphate is converted into glucose 6 phosphate. Glucose 6 phosphate makes the body more active or has a stimulant effect (Ferreira et al., 2015).

The mechanism of action of caffein in increasing stamina is by inhibiting the phosphodiesterase enzyme and can also block adenosine receptors which work in the opposite way to caffeine. Adenosine has great influence on nerve cell activity (Winata, 2016).

Secondary metabolite compounds in pepper include alkaloids, flavonoids, tannins, saponins, glycosides and essential oils. Physiologically, they can increase blood circulation by improving body activity so that it will indirectly improve organ function by increasing the motor activity of experimental animals and fatigue threshold value (Sumarny et al., 2013).

Flavonoids can provide a tonic effect by working as adenosine A_1 receptor antagonists which play a role in sleep regulation. Flavonoids such as isoflavones will eliminate drowsiness and fatigue, thereby producing freshness and enthusiasm in the body (Annisa et al., 2022).

Table 4. Results of the stimulant activity test of white paper extract

Treatment	Sample	Average Increase in stamina (%)
1	White pepper extract (0.32 mg/20 g BW)	63.92 ± 26.01*
2	White pepper extract (0.65 mg/20 g BW)	120.81 ± 24.01*
3	White pepper extract (1.3 mg/20 g BW)	179.21 ± 25.08**
Control (+)	Caffein (0.26 g/20 g BW)	232.55 ± 85.89**
Control (-)	CMC-Na 0.5%	21.10 ± 2.78*

* ($p<0.05$)
** ($p>0.05$)

CONCLUSIONS

Simplicia of white pepper was positive for containing secondary metabolism of flavonoids, alkaloids, saponins, tannins and triterpenoids. The 96% ethanol extract of white pepper fruit at a dose of 1.3 mg/20 g BW had the most active stimulant activity with an average value % increase in stamina of 179.31% which was not

significanly different from the positive control caffeine.

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AUTHORS' CONTRIBUTIONS

Conception and study design were done by Arini Khaerunnisa, Dhyneu Dwi Jayantie and Nani Suryani; all authors provided administrative technical/logistic support and conducted data collection and assembly; data analysis and interpretation were done by Arini Khaerunnisa, Nani Suryani and Sutihat; critical revision of the article for important intellectual content was done by Arini Khaerunnisa, Dhyneu Dwi Jayantie and Nani Suryani; all authors contributed to article drafting and approved final version.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

ETHICAL CONSIDERATION

Ethical issues (including plagiarism, data fabrication, double publication, etc) have been completely observed by the authors

BIBLIOGRAPHY

Ahwan, A. (2018). Identification and Isolation of Non Polar, Semipolar and non polar isolate from hexana fraction of ethanol extract of belt leaves (*Piper betle* L.) using TLC Scanner and GC-MS Methods. Jurnal Ilmiah Farmasi, 1(2), 88–99. https://doi.org/10.29313/jiff.v1i2.3746

Annisa, F. N., Fauziyyah, G. F., Wicaksono, I. A., & Lestari, K. (2022). Review Article: Potential of Herbal Plants in Indonesia in Improving Body Fitness. Jurnal Ilmiah Farmasi Farmasyifa, 5(2), 161–182. https://doi.org/10.29313/jiff.v5i2.8841

Ashokkumar, K., Murugan, M., Dhanya, M. K., Pandian, A., & Warkentin, T. D. (2021). Phytochemistry and therapeutic potential of black pepper [*Piper nigrum* (L.)] essential oil and piperine: a review. Clinical Phytoscience, *7*(1). https://doi.org/10.1186/s40816-021-00292-2

Boangmanalu, K. R., & Zahrotun, A. (2018). Potential medicinal effect of piper plants: *Piper nigrum* L., *Piper retrofractum* Vahl., *Piper betle* Linn., *Piper cubeba* L. dan *Piper crocatum* Ruiz & Pav. Farmaka, 16(2), 204–212. https://doi.org/10.24198/jf.v16i3.17699

Damanhouri, Z. A. (2014). A Review on Therapeutic Potential of *Piper nigrum* L. (Black Pepper): The King of Spices. Medicinal & Aromatic Plants, 03(03). https://doi.org/10.4172/2167-0412.1000161

Efia, R. (2019). *Stimulant effect of a combination of ethanol extract of black pepper (Piper nigrum L.) and red ginger (Zingiber officinale Roscoe) on male mice*. Pakuan University.

Febrianasari, N., Wijayanti, R., & Apriadi, A. (2016). Stimulant Test of Garlic Bulb (*Allium sativum* L.) Extract on Swiss Webster Mice. Jurnal Farmasi Sains Dan Praktis, 1(2), 42–49. https://doi.org/10.31603/pharmacy.v1i2.229

Ferreira, L. G., Dos Santos, R. N., Oliva, G., & Andricopulo, A. D. (2015). Molecular docking and structure-based drug design strategies. In *Molecules* (Vol. 20, Issue 7). https://doi.org/10.3390/molecules200713384

Fithriyana, R. (2018). Factors Related to the Incident of Dypepsia in Patients in the Working Area of Bangkinang Health Center. Jurnal Kesehatan Masyarakat, 2(2), 43–54. https://doi.org/10.31004/prepotif.v2i2.79

Gorgani, L., Mohammadi, M., Najafpour, G. D., & Nikzad, M. (2017). Piperine—The Bioactive Compound of Black Pepper: From Isolation to Medicinal Formulations. Comprehensive Reviews in Food Science and Food Safety, 16(1), 124–140. https://doi.org/10.1111/1541-4337.12246

Hasibuan, A. S., & Edrianto, V. (2021). Phytochemical screening of ethanol extract of red onion tubers (*Allium cepa* L.). Jurnal Pengmas Kestra (Jpk), 1(1), 80–84. https://doi.org/10.35451/jpk.v1i1.732

Herwana, E., Pudjiadi, L. L., Wahab, R., Nugroho, D., & Hendrata, T. (2005). The effect of giving stimulant drinks on fatigue in rats. Jurnal Universa Medicina, 24(1).

Hikmawanti, N. P. E., Hariyanti, H., Aulia, C., & Viransa, V. P. (2016). The Content of Piperine in Black and White Pepper Fruits (*Piper nigrum* L.) Extracted with Variation of Ethanol concentrations using TLC-Densitometry Method. Media Farmasi: Jurnal Ilmu Farmasi, 13(2), 173. https://doi.org/10.12928/mf.v13i2.7769

Isnenia. (2017). Test Effect of Wooden Sappan Infusa Stimulant toward Male Mice. Jurnal Analis Farmasi, 2(2), 146–154. https://doi.org/10.33024/jaf.v2i2.1175

Katzung, B. . (2014). *Basic and Clinical Pharmacology XII Edition*. EGC.

Levita, J., Simanjuntak, N., & Subarnas, A. (2019). Review: Included Biopiperine and Anti-inflammatory Activity of Piperine. Jurnal Sains & Teknologi Farmasi Indonesia, 8(1). https://doi.org/10.58327/jstfi.v8i1.94

Mustopo, L. W. (2011). Aviation safety and the psychological aspects of "fatigue." Psikobuana, 3(2), 126–134.

Rahimah, S. B., Djunaedi, D. D., Soeroto, A. Y., & Bisri, T. (2019). The phytochemical screening, total phenolic contents and antioxidant activities in vitro of white oyster mushroom (*Pleurotus ostreatus*) preparations. Open Access Macedonian Journal of Medical Sciences, 7(15), 2404–2412. https://doi.org/10.3889/oamjms.2019.741

Rahmi, M., Tobat, S. R., & Ningsih, S. (2020). Testing The Stimulant Effects of the Central Nervous System of South African Leaf Ethanol Extract (*Vernonia amygdalina* Delile) on Female White Mice. MEDFARM: Jurnal Farmasi Dan Kesehatan, 9(2), 55–62. https://doi.org/10.48191/medfarm.v9i2.35

Ridwan, E. (2013). Ethics of Using Experimental Animals in Health Research. J Indon Med Assoc, 63, 112–118.

Setyowati, D. L., Shaluhiyah, Z., & Widjasena, B. (2014). Causes of Work Fatigue in Furniture Workers Factors. Kesmas: National Public Health Journal, 8(8), 386. https://doi.org/10.21109/kesmas.v8i8.409

Sidrotullah, M., Pahmi, K., & Amrillah. (2019). The Effect of Palm Sugar Decoction (*Arenga Pinnata*) Treatment Given by Perolar on the Stamina of Mice Using the Natatory Exhaustion Method. Jurnal Ilmu Farmasi, 7(2), 83–86. https://doi.org/10.51673/jikf.v7i2.585

Soemarie, Y. B., Astuti, T., & Rochmah, N. (2017). Formulation of Avocado Leaf Ethanol Extract Oinment (Persea americana Mill) as Antiacne. Jurnal Ilmiah Manuntung, 2(2), 224–232. https://doi.org/10.51352/jim.v2i2.70

Sumarny, R., Rahayu, L., Made, N. I., & Sandhiutami, D. (2013). Stimulant effect of infusion of black pepper (*Piperis nigri fructus*) in mice. Jurnal Ilmu Kefarmasian Indinesia, 11(2), 142–146. jifi.ffup.org/

Syafitri, N. E., Bintang, M., & Falah, S. (2014). Phytochemical Content, Total Phenols, and Total Flavonoids of Harendong Fruit Extract (*Melastoma affine* D. Don). Current Biochemistry, 3(3), 105. https://journal.ipb.ac.id/index.php/cbj/article/view/17419

Syhadat, A., & Siregar, N. (2020). Phytochemical Screening of Katuk (*Sauropus androgynus*) Leaves as a Breast Milk Promotor. Kesehatan Ilmiah Indonesia, 5(1), 85–89. http://dx.doi.org/10.51933/health.v5i1.246

Tary, N. L. N., & Sujono, T. A. (2018). Effect Test Stimulant of Curcuma Rhizome Infusion (*Curcuma xanthorrhiza* Roxb.) On Male Mice Swiss Strain. Pharmacon: Jurnal Farmasi Indonesia, 14(1),

14–18. https://doi.org/10.23917/pharmacon.v14i1.540

Uthia, R., Arifin, H., & Efrianti, F. (2017). The Effect of Fractionation Results of Basil Leaf extract (*Ocimum sanctum* L.) on Central Nervous System Activities in Male White Mice. Farmasi Higea, *9*(1), 85–95. http://dx.doi.org/10.52689/higea.v9i1.161

Wade, C., & Travis, C. (2008). *Psychology, 9th Edition Volume 1*. Erlangga.

Wendersteyt, N. V., Wewengkang, D. S., & Abdullah, S. S. (2021). Antimicrobial Activity Test of Exstracts And Fractions of Ascidian Herdmania Momus From Bangka Island Waters Likupang Against The Growth Of *Staphylococcus aureus, Salmonella typhimurium,* And *Candida albicans.* Pharmacon, 10(1), 706. https://doi.org/10.35799/pha.10.2021.32758

Winata, S. D. (2016). Symptoms, Diagnosis, and Treatment in Caffeine Drinking Patients Who Experience Addiction. Jurnal Kedokteran Meditek, 21(57), 1–7. https://doi.org/10.36452/jkdoktmeditek.v21i57.1168