

POTENTIAL OF FERMENTED SINGLE CLOVES GARLIC (*Allium sativum* L.) IN INHIBITING DEFECTS IN PREGNANT WHITE MICE EXPOSED TO CIGARET SMOKE

Wahyu Adji Prasetya¹, Hayat², Kriana Effendi^{3*}

Faculty of Pharmacy and Science
Muhammadiyah University Prof. DR. Hamka, East Jakarta
Correspondence email: kriana_effendi@uhamka.ac.id

ABSTRACT

The antioxidant content of single garlic (*Allium sativum* L.) preparations is thought to be able to inhibit the negative impacts resulting from exposure to cigarette smoke on the fetus. The negative impacts of cigarette smoke on the fetus are low birth weight and fetal death. This study aims to observe the effect of administering single garlic preparations on inhibiting fetal defects and weight gain exposed to cigarette smoke during pregnancy. The test was conducted by dividing 25 pregnant mice into 5 groups, each consisting of 5 mice, negative control (exposed to cigarette smoke), positive control (exposed to cigarette smoke and silymarin preparations), dose I 10.27 mg/20 gBB, dose II 20.54 mg/20gBB, dose III 41.08 mg/20gBB. The parameters measured included fetal weight, fetal defects, and number of fetuses. The average fetal weight data were analyzed statistically using one-way ANOVA with a significance value of <0.0001 then continued with the Tukey test. The results showed that all test groups of single garlic preparations could significantly inhibit fetal defects compared to negative controls. Dose III 41.08 mg/20gBW is an effective dose in inhibiting fetal defects.

Keywords: *Allium sativum* L., cigarette smoke, defects, fetus.

INTRODUCTION

The leading cause of death in the world is smoking. Early death and disability are the main risk factors of smoking. WHO estimates that 1.1 billion people smoke worldwide, representing one-third of the global population. There are around 3 million people who die from smoking each year worldwide, and this number will continue to rise to 10 million each year by 2030, with 70% of these deaths occurring in developing countries. Indonesia is the third country with the highest number of smokers in the world, after China and India. Compared to the Philippines, Thailand, and Myanmar, Indonesia has the highest mortality rate with 194 thousand (Sampurna Sampurna & Siti Thomas Zulaikhah, 2022). Many smokers do not pay attention to the environment where they dispose of their smoke, resulting in many people being exposed to cigaret smoke.

Cigaret smoke contains various chemicals, including nicotine, tar, and carbon monoxide (CO). Tar is a product that is produced when cigarets are burned and is carcinogenic. Tobacco plants naturally contain a chemical called nicotine. These particles are a mixture of thousands of components of smoke, not just from one chemical (Nurhayati et al., 2020). One of the people exposed to cigaret smoke is pregnant women, who are more vulnerable to the negative effects of cigaret smoke exposure on the fetus they are carrying (Choirunnisa et al., 2022). In addition to being a passive smoker, smoking during pregnancy also has very detrimental effects on pregnancy because it can hinder fetal development, cause low birth weight, premature birth, and increase the risk of maternal and infant mortality (Hanum & Wibowo, 2020).

The substances found in cigarets, such as nicotine and carbon monoxide (CO), are two components in cigaret smoke that are harmful to pregnant women. Nicotine causes vasoconstriction, reducing blood flow to the fetus thru the umbilical cord, which decreases the fetus's ability to receive the nutrients it needs (Safitri & Syahrul, 2015). Carbon monoxide (CO) inhibits hemoglobin from binding oxygen, causing the fetus to lack oxygen and nutrients. As a result, the growth and development of the fetus during pregnancy are hindered due to exposure to cigaret smoke (Andini & Zayani, 2022).

Cigaret smoke contains complex compounds that contain many free radicals and oxidants, which can lead to a decrease in antioxidant concentration, an increase in oxidative stress, and an increased risk of several chronic diseases (Zulaikhah et al., 2021). Free radicals are molecules with unpaired electrons and very high reactivity that are produced during normal cell metabolism or can be obtained externally (Sinaga, 2014). The body can naturally produce free radicals thru respiration in the

Proceeding books:

The 3rd International Conference and Innovation Exhibiti on Global Education (ICEGE)

mitochondria, inflammatory reactions, and excessive food consumption because during the metabolic process, it not only generates energy but also produces free radicals. Free radicals that originate from the environment include exposure to UV sunlight, car emissions, and cigaret smoke. Oxidative stress is a condition where free radicals and antioxidants are imbalanced, caused by a lack of antioxidants and excessive production of free radicals (Fadiyah et al., 2018). Oxidative stress can be prevented by consuming foods high in antioxidants, including vitamin C, vitamin E, vitamin A, fruits, and vegetables. Antioxidants have the ability to protect cells from damage caused by free radicals (Nursyahrina & Lestari, 2021).

Research Tools and Materials

Research Tools

The tools that will be used in this research include animal cages, animal scales, analytical scales, a smoking chamber, mouse drinking bottles, stirring rods, measuring cylinders (Pyrex), spatulas, oral sondes, syringes, dropper pipettes, mortars, pestles, test tubes, watch glasses, surgical instruments, and glassware.

Research Materials

Test Materials

The test material to be used in this research is a preparation of Single Clove Garlic (*Allium sativum* L.) obtained from the producer SAB, Bekasi. The material was identified at BRIN (National Research and Innovation Agency).

Chemical Materials

The chemical materials that will be used in this research are Boin's solution (14% formaldehyde, saturated picric acid, glacial acetic acid), Mayer's reagent, Bouchardat's reagent, 10% gelatin, glacial acetic acid, 2N HCl, NaOH, Mg powder, Na CMC, 1% FeCl₃, concentrated H₂SO₄, aquadest, ketamine, and unfiltered clove cigarets.

Inducing Agent

The inducing agent to be used is kretek cigarets, 2 times a day, 2 cigarets. Given at 09:00 AM and 03:00 PM Western Indonesia Time (WIB).

Research Procedure

1. Material Determination

Determination is conducted at BRIN (National Research and Innovation Agency) to verify the accuracy of the samples in the research to ensure they meet the expected standards.

2. Preparation of Single Clove Garlic

Single clove garlic (*Allium sativum* L.) is wrapped using aluminum foil in two or three layers. After that, place the single clove garlic wrapped in aluminum foil into the rice cooker and close it. Then, set the rice cooker to the warm temperature (70-80°C), and wait for 7 days (Romsiah et al., 2020).

3. Phytochemical Screening of Single Clove Garlic Preparation (*Allium sativum* L.)

a. Alkaloid Identification

A 1 g sample is weighed and added to 5 mL of aquadest, heated for 2 minutes, then cooled and filtered. The filtrate is added to 2 mL of Mayer's and Dragendorff's reagents, and 2 mL of Bouchardat's reagent is added to the filtrate. If a white precipitate forms with Mayer's reagent or a red precipitate with Dragendorff's reagent, and a brown precipitate with Bouchardat's reagent, then alkaloids are present (Hanani, 2015).

b. Identification of Flavonoids

A 1 g sample is weighed and added to 2 mL of ethanol, then heated and filtered. The filtrate is added with Mg metal and 10 drops of concentrated HCl, if a red to purple-red, yellow, or orange color forms, then flavonoids are present (Hanani, 2015).

c. Identification of Tannins

A 1 g sample is weighed and added to 70% ethanol solvent, then heated for 5 minutes, cooled, and filtered. The filtrate is added with 1-3 drops of 10% gelatin, if a white precipitate forms, then tannins are present (Hanani, 2015).

d. Saponin Identification

A 1 g sample is weighed and added to 10 mL of distilled water, then 1 drop of 2N HCl is added. Shake vigorously for 10 seconds; if stable foam forms, saponin is present (Hanani, 2015).

e. Identification of Terpenoids and Steroids

Proceeding books:

The 3rd International Conference and Innovation Exhibiti on Global Education (ICEGE)

A 1 g sample is weighed and added to 10 drops of anhydrous acetic acid and 3 drops of concentrated sulfuric acid. The positive reaction test for terpenoids is indicated by the formation of a golden yellow solution, and the formation of a green color indicates a positive result for steroids (Aina et al., 2024).

4. Examination of the Characteristics of Single Garlic Preparation (*Allium sativum* L.)

a. Organoleptic Examination

The organoleptic examination is conducted using the five senses, including the examination of shape, color, smell, and taste of the single garlic preparation.

5. Preparation of Test Animals

a. Acclimatization

The test animals were acclimated in cages for approximately 7 days to allow them to adapt to the new environment. During this period, estrus observation was conducted twice, along with daily weight measurements. The test animals were divided into 5 groups, with each group consisting of 5 test animals (Monica, 2018).

b. Grouping of Test Animals and Treatment

This research was conducted experimentally with a completely randomized design, using 25 male and female mice divided into 5 groups, each consisting of 5 pregnant female mice:

6. Determination of Estrus Phase in Female Mice

The estrus phase in female mice is characterized by swelling around the vagina, as well as a clearly open, moist vagina with reddish mucus. It is during this phase that the mice can be mated because the female mice are willing to mate during this phase.

7. Mating Experimental Animals

The mating of test animals is carried out by placing the male animal into the cage of the female animal that is already in estrus.

8. Allocation of Pregnant Animals

Pregnant female animals are kept in individual cages and randomly grouped with numbering so that the mice used can represent the entire population.

9. Dose Calculation In this study

the subjects were divided into 5 groups consisting of a negative control, a positive control, a dose I group, a dose II group, and a dose III group.

a. Dose of single clove garlic

In the preparation of single garlic, a human dose of 5000mg/60kg body weight is used, which is then converted into a mouse dose first.

Dose conversion formula based on HED (mg/kgBW):

Mouse dose = Human dose x (Human KM)/(Mouse KM). ... (1)

Mouse dose = 83.3 mg/kgBW x 37/3

Mouse dose = 1027 mg/kgBW

To determine the effective concentration of single clove garlic preparation in inhibiting defects in pregnant white mice exposed to cigaret smoke, this study applied three different doses:

Dose 1 = $\frac{1}{2} \times 1027 \text{ mg/kgBW} = 513.5 \text{ mg/kgBW} = 10.27 \text{ mg/20gBW}$

Dose 2 = $1 \times 1027 \text{ mg/kgBW} = 1027 \text{ mg/kgBW} = 20.54 \text{ mg/20gBW}$

Dose 3 = $2 \times 1027 \text{ mg/kgBW} = 2054 \text{ mg/kgBW} = 41.08 \text{ mg/20gBW}$

b. Silymarin dosage

In previous research, the dosage of silymarin used on rats as an antioxidant was 100 mg/kgBW (Ramadhani, 2020).

Mice dose = Rat dose x (Rat body weight)/(Mouse body weight)..... (2)

Mice dose = 100 mg/kgBW x 6/3

Mice dose = 200 mg/kgBW = 4 mg/20gBW

c. Ketamine dose

The ketamine dose for humans intramuscularly is 6.5 mg/kgBW (Association, 2015).

Mice dose = Human dose x (Human body weight)/(Mouse body weight).... (3)

Proceeding books:

The 3rd International Conference and Innovation Exhibiti on Global Education (ICEGE)

Mice dose = $6.5 \text{ mg/kgBW} \times 37/3$

Mice dose = $80.16 \text{ mg/kgBW} = 1.60 \text{ mg/20gBW}$

10. Preparation of Test

a. Solution Preparation of Na-CMC

Suspension Preparation of Na-CMC solution with a concentration of 0.5%. Weigh 500 mg of Na-CMC, then sprinkle it over 10 mL of hot water, and let it swell. Add aquadest up to 100 mL, then grind vigorously until homogeneous.

b. Preparation of test substance

The preparation of single garlic extract was applied in 3 different dose variations by doubling and halving the reference dose of 1027 mg/kgBW.

- (1) Dose I (513.5 mg/kgBW) The single garlic extract was prepared with a concentration of 20.54 mg/mL. 308.1 mg of single garlic was weighed and then dissolved with 0.5% Na-CMC to a total volume of 15 mL.
- (2) Dose II (1027 mg/kgBW) The single garlic extract was prepared with a concentration of 41.08 mg/mL. 616.2 mg of single garlic was weighed and then dissolved with 0.5% Na-CMC to a total volume of 15 mL.
- (3) Dose III (2054 mg/kgBW) The single garlic extract was prepared with a concentration of 82.16 mg/mL. 1,232 mg of single garlic was weighed and then dissolved with 0.5% Na-CMC to a total volume of 15 mL. $\text{VAO (mL)} = (\text{Dose (mg/kg)} \times \text{BW (kg)}) / (\text{concentration (mg/mL)}) = \dots\dots\dots(4)$

c. Preparation of comparison substance

The silymarin suspension was prepared with a concentration of 8 mg/mL. 120 mg of silymarin powder was weighed and then dissolved with 0.5% Na-CMC to a total volume of 15 mL.

$\text{VAO (mL)} = (\text{Dose (mg/kg)} \times \text{BW (kg)}) / (\text{concentration (mg/mL)}) = \dots\dots\dots(5)$

11. Process of Cigaret

Smoke Exposure The test animals are placed in a special cage (smoke chamber) that has one hole at the top to introduce cigaret smoke exhaled from a 50 cc syringe until the smoke is completely inhaled by the mice. The cigaret smoke used comes from one clove cigaret, which is exposed twice a day, morning and evening, by lighting the cigaret, inhaling it with a 50 cc syringe, and then exhaling it into the smoke chamber.

12. Laparotomy

Laparotomy is a procedure to extract the fetus from the pregnant mouse thru an incision in the abdominal area (Monica, 2018). Laparotomy is performed on the 18th day of pregnancy to retrieve the fetus. Before the laparotomy, the mouse is anesthetized using a ketamine injection.

13. Fixation

After being observed visually, the fetus is then fixed using Bouin's solution to turn it yellow and make it easier to section (Monica, 2018).

RESEARCH METHOD

The average fetal weight data and the number of fetuses taken from various groups underwent normality and homogeneity tests. Once the data was obtained, it was continued with the one-way ANOVA method. If there were significant differences between several groups, it was followed by the Tukey HSD test.

RESULTS AND DISCUSSION

A. Plant Determination

Results Determination is aimed at ensuring and providing accurate information regarding the plants used in this research. The determination results from BRIN (National Research and Innovation Agency), Cibinong, West Java, indicate that the sample used in this study is a single bulb garlic plant (*Allium sativum* L.) from the Amaryllidaceae family. The determination results can be seen in Appendix

Proceeding books:

The 3rd International Conference and Innovation Exhibiti on Global Education (ICEGE)

B. Results of Phytochemical Screening of Single Clove Garlic (*Allium sativum* L.) a. Results of Organoleptic Examination

Table 2. Results of Organoleptic Examination of Single Clove Garlic Preparation (*Allium sativum* L.)

a. Results of Organoleptic Examination

Organoleptic Test				
Type	Shape	Smell	Flavour	Colour
Sediaan	Suspensi	Special	special	Brown

b. Results of Phytochemical Screening of Single Clove Garlic (*Allium sativum* L.) The phytochemical screening test on single clove garlic aims to determine the chemical compounds contained in single clove garlic preparations, including alkaloid test, flavonoid test, saponin test, steroid test, and tannin test. The results of the phytochemical screening can be seen in Table 3.

No	Filtering	Results
1	Alkaloid	+
2	Flavonoid	+
3	Saponin	+
4	Tanin	+
5	Steroid	-
6	Terpenoid	+

Explanation:

(+) : Positive for containing compounds

(-) : Negative for containing compounds

The results of the phytochemical screening test indicate that the compounds contained in the single clove garlic preparation are alkaloids, flavonoids, saponins, and tannins, while steroids were not detected because steroids are positive for non-polar solvents. This is in line with the research by Pudiarifanti & Farizal (2022). Alkaloids are compounds found in plant tissues, which play an important role in regulating metabolism and plant development, and are mostly derived from angiosperms. In the phytochemical screening test on single bulb garlic, the results were positive for alkaloids because during the Dragendorff, Mayer, and Bouchardat tests, the results were positive. Alkaloids in plants can function to protect against insects, regulate growth, and store nutrients (Maisarah et al., 2023). Flavonoid compounds have several functions, including as antioxidants, antimicrobials, plant antivirals, anti-HIV, antitumor agents, and flavonoid compounds can also inhibit the growth of cancer cells. Flavonoids are identified by adding magnesium powder and concentrated HCl. This process aims to reduce the benzopyrone core present in the flavonoid structure, resulting in the formation of a red-colored flavillium salt, which is an indicator of the presence of flavonoids (Andika et al., 2020). The main compound that actively acts as an antioxidant is the phenolic compound, such as flavonoids. This compound contains hydroxyl groups that can donate hydrogen atoms to free radicals, making flavonoids potential antioxidants (Tuslinah et al., 2023).

Saponin compounds play an important role as a defense system in plants. Saponin identification is carried out by adding hot distilled water to the filtrate, which is then shaken to form foam. A positive result is obtained if the foam remains stable when 2N HCl is added (Octariani et al., 2021). The identification of tannin compounds was carried out by adding 10% gelatin. The identification of tannins that formed a white precipitate indicates that the compound positively contains tannin compounds

Proceeding books:

The 3rd International Conference and Innovation Exhibiti on Global Education (ICEGE)

(Bariyyah

et

al.,

2018).

The identification of terpenoids yielded a positive result, indicated by a color change to golden yellow, while the identification of steroids yielded a negative result as there was no color change.

C. Results of Single Garlic Preparation Test on Mouse Fetus

The administration of single garlic preparation on the development of pregnant mouse fetuses exposed to cigaret smoke was conducted from day 6 to day 14 because during this period, the mice were in the organogenesis phase, making them susceptible to free radicals. The observed results included the morphology of the mouse fetuses, which were then fixed using Bouin's solution for 14 days. The observed results in the morphology of the mouse fetuses included their completeness and abnormalities. The administration of single garlic preparation showed that there were no defects in all test animal groups.

D. Results of Observation on Mouse Fetuses

The results of the observation on the number of fetuses showed that the number of mouse fetuses in the dose III preparation was higher compared to the dose I preparation, dose II preparation, negative control, or positive control. During the observation, 5 dead fetuses, 10 undeveloped or resorbed fetuses, and 2 fetuses with abnormalities were found in the negative control group. The observed defect parameters include weight loss, which indicates growth and development disturbances in the mouse fetus. Another observed defect parameter is the number of fetuses and cleft palate in the fetuses. In addition, other parameters such as resorption and death were found in the negative control group. From the observations that have been conducted, average weight data was obtained. After the cleft palate was performed on the fetus, no defects were found in all test groups.

Table 4. Fetal Defect Parameters

Treatment	Total	Defect Parameter			Kleft
Group	Number	Dead	Resorpsi	physical	Palatte
	of Fetus	Fetus		disability	
Kontrol	46	5	10	2	Normal
Negatif					
Kontrol	48	-	-	-	Normal
Positif					
Dosis I	47	-	-	-	Normal
Dosis II	45	-	-	-	Normal
Dosis III	49	-	-	-	Normal

The One Way ANOVA statistical test in this study used GraphPad Prism software. The results of the One Way ANOVA calculation indicate a significant treatment effect on fetal body weight. The significance value obtained was <0.0001. The Tukey test is used as a continuation of the One Way ANOVA analysis to determine the location of the differences. In the Tukey test, significant differences were found between the negative control and the positive control and treatment groups. The fetal body weight obtained in the negative group was lower compared to the positive control, dose I, dose II, and dose III.

E. Discussion

This study aims to determine the potential of fermented single bulb garlic (*Allium sativum* L.) preparations on pregnant white mice exposed to cigaret smoke in inhibiting defects caused by cigaret smoke exposure to mouse fetuses. The initial stage of this research begins with determination, which is the process of identifying the plant used to ensure the accuracy of the plant used. Based on the results of the determination analysis, the plant used is identified as single bulb garlic (*Allium sativum* L.) from the Amaryllidaceae family. The fermentation of single bulb garlic has natural antioxidant properties that can counteract free radicals. However, after undergoing the fermentation process into black garlic, its

Proceeding books:

The 3rd International Conference and Innovation Exhibiti on Global Education (ICEGE)

bioactive content undergoes significant changes that result in increased antioxidants. The IC₅₀ values of single clove garlic and black garlic in the same extraction solvent provide the same antioxidant activity, but the resulting IC₅₀ values are different, where the IC₅₀ value of black garlic is smaller compared to single clove garlic, thus the antioxidant activity of black garlic is stronger. Single clove garlic and black garlic positively contain flavonoid compounds, but the concentration of these compounds in single clove garlic and black garlic may differ, with more antioxidant potential compounds found in black garlic (Tuslinah et al., 2023).

The test animals used in this study were 25 female white mice. Before the treatment, the test animals were first acclimated to ensure they could adapt to the environment, and the estrus phase was observed. The estrus phase is characterized by an open, moist vagina and swelling in the vaginal area of the mice. The estrous cycle of mice lasts for 4 days (Fatmala et al., 2022). The test animals were mated in the evening with a ratio of 1 male mouse to 4 female mice. Successful mating in mice can be observed by the presence of a vaginal plug, which is a clump of fluid that covers the mouse's vaginal opening (Effendy, 2017). Mice that have become pregnant are marked by the observation of a vaginal plug as an indicator of day 0 of pregnancy. This vaginal plug is visible in the morning. If the vaginal plug is observed, it can be determined as day 0 of pregnancy.

This study is divided into 5 groups, namely the negative group, positive group, dose I group, dose II group, and dose III group, with each group consisting of 5 individuals. The negative control was only exposed to cigaret smoke, the positive control was exposed to cigaret smoke and given silymarin preparation, and the dose groups were exposed to cigaret smoke and given single garlic preparation with doses of 10.27 mg/20gBB, 20.54 mg/20gBB, and 41.08 mg/20gBB. The exposure to cigaret smoke in the negative control, conducted twice a day with 2 cigarets per exposure, resulted in a significantly lower fetal body weight compared to the positive control and dose groups. Research conducted by (K. S. D. Lestari et al., 2015) shows that cigaret smoke contains free radicals that can induce oxidative stress, thereby hindering nutrient distribution and affecting fetal development. In addition, fetal death was also observed in the negative control. Fetal death is likely caused by oxidative stress, which can lead to pregnancy issues such as placental dysfunction and apoptosis (Medika et al., 2016).

Negative control found 10 resorbed fetuses, which was caused by the loss of cell totipotency during the organogenesis period, resulting in the cells being unable to repair damage and inhibiting further development, so the mouse fetuses died and only red clots were formed. Additionally, the nicotine content in cigaret smoke can penetrate the placenta, leading to resorption (Medika et al., 2016).

Positive control, the test animals were exposed to cigaret smoke and then given a comparison preparation, namely silymarin. In this positive control, there was a decrease in fetal weight, but it was not as significant compared to the negative control and the dose I and II groups. The dose I, dose II, and dose III groups, after being exposed to cigaret smoke, were each given a single garlic preparation with different doses. The results showed that in the dose I and II groups, there was a decrease in fetal weight and size compared to the positive control and the dose III group. The weight loss in the dose I and II groups occurred because the single garlic preparation at doses I and II was too small, making it not very effective in inhibiting the adverse effects caused by cigaret smoke exposure. The single garlic preparation contains flavonoids that act as antioxidants, which can enhance antioxidant enzymes to counteract free radicals caused by cigaret smoke exposure. Flavonoids can address oxidative stress by inhibiting lipid peroxidation (Amida et al., 2021).

Weight loss is one of the observed parameters of deformity, as it can indicate growth and development retardation in the fetus. Other parameters observed include the number of fetuses and cleft palate in the fetuses. In addition to weight loss, the number of fetuses, and cleft palate, resorption and death were also found in the negative control. Based on these parameters, average body weight data was obtained. The average body weight data of the fetus was analyzed using the One Way ANOVA test, yielding ($p < 0.0001$). This study was continued with the Tukey test to determine significant differences between each test group. The fetal body weight in the negative control was smaller compared to the positive control and the dose groups.

CONCLUSIONS

Based on the research results that have been conducted, it can be concluded that the use of single clove garlic preparation (*Allium sativum* L.) is capable of inhibiting defects caused by exposure to cigaret smoke. The results of the Tukey test show that dose III 41.08 mg/kgBW has the most significant

Proceeding books:

The 3rd International Conference and Innovation Exhibiti on Global Education (ICEGE)

ability to inhibit defects, so it can be concluded that dose III is the effective dose for inhibiting defects caused by exposure to cigaret smoke.

REFERENCES

- Aina, N., Saleh, C., & Marlina, E. (2024). REVIEW ARTICLE: PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL ACTIVITIES OF KRATOM PLANT (*Mitragyna speciosa* Korth) 156–159.
- Amida, N., Lisdiana, L., Christijanti, W., & Iswari, R. S. (2021). The effect of black garlic extract on the quality of rat spermatozoa after exposure to cigaret smoke. *Proceedings of the 9th National Biology Seminar*, 9, 298–303.
- Andika, B., Halimatussakdiah, H., & Amna, U. (2020). Qualitative Analysis of Secondary Metabolite Compounds from Siam Weed Leaf Extract (*Chromolaena odorata* L.) in Langsa City, Aceh. *QUIMICA: Journal of Chemical Science and Applied*, 2(2), 1–6.
- Andini, V., & Zayani, N. (2022). The Effect of Zuriat Fruit Tea (*Hyphaenethebeica*) on the Reproductive Performance of Mice (*Mus musculus*). *Jurnal Nusantara Hasana*, 2(1), 44–50.
- Angela, E., Kunaedi, A., Suharyani, I., & Tinggi Farmasi Muhammadiyah Cirebon, S. (2022). The Effect of Honey Fermentation Time with Single Garlic (*Allium sativum* L.) on the Inhibition of *Propionibacterium acnes* Bacteria. *Open Journal Systems STF Muhammadiyah Cirebon: Ojs.Stfmuhammadiyahcirebon. Ac.Id*, 7(3), 407–418.
- Asmal, A. (2021). Determination of Vitamin C Content in Single Clove Garlic (*Allium sativum* Linn) Using Iodimetry Method Submitted as One of the Requirements to Obtain a Diploma (DIII) in the Pharmacy Study Program at Poltekkes Kemenkes Bengkulu By: MERLIE WULANDARI YEAR. 2, 22–29.
- Ayuningtyas, M. P. (2021). The Effect of Ethanol Extract of Red Spinach Leaves (*Amaranthus tricolor* L.) on the Number of Mice (*Mus musculus*) Exposed to Cigaret Smoke.
- Bariyyah, S. K., Hanapi, A., Fasya, A. G., & Abidin, M. (2018). ANTIOXIDANT ACTIVITY TEST AGAINST DPPH AND IDENTIFICATION OF ACTIVE COMPOUND GROUPS IN ROUGH EXTRACTS OF *Chlorella* sp. MICROALGAE CULTIVATED IN SPROUT EXTRACT MEDIUM. *Alchemy*, 2(3), 195–204.
- Choirunnisa, A., Febriyana, F., Sari, E. T. P., Ambarwati, N. M., & Nurdiantami, Y. (2022). The Influence of Cigaret Smoke on Pregnant Women: A Literature Review. *Tambusai Health Journal*, 3(2), 183–192.
- Denny, R., Yudistira, A., & Mpila, D., A. (2022). Antioxidant Activity Test of Ethanol Extract of *Stylissa carteri* Sponge from Mentehage Island, North Minahasa. *Pharmacon*, 11(1), 1309–1314.
- Effendy, M. C. O. S. (2017). COMPARISON OF PREGNANCY RATE BETWEEN MULTIPAROUS MICE MATED OVERNIGHT WITH AND WITHOUT ESTRUS SYNCHRONIZATION.
- Fadiyah, A. F., Wardhani, R. M., Rahmatika, N., & Wijayanti, S. P. M. (2018). Exploration of the Potential of Liquid Extracts from Kecombrang Leaves Containing Antioxidants as Neutralizers of Free Radicals in the Blood of Gas Station Attendants. *Jurnal Litbang Kota Pekalongan*, 15, 8–16.
- Fatmala, Y., Mahrus, M., & Zulkifli, L. (2022). Efectos estrogénicos del extracto metanólico de hojas de Tapak Dara (*Catharantus roseus*) en el ciclo estral de ratonas adultas (*Mus musculus*) de la cepa Balb/C. *Jurnal Biologi Tropis*, 22(3), 803–808.
- Hanum, H., & Wibowo, A. (2020). The Influence of Environmental Cigaret Smoke Exposure on Pregnant Mothers on the Incidence of Low Birth Weight Infants. *Unila Medical Journal*, 5(5), 2.
- Haryanto, Pertiwi, W., & Ihsani, N. (2019). The Estrous Cycle of Virgin Female Mice (*Mus musculus*) Strain BALB/c after Exposure to Various Types of Sound. *Jurnal Sains, Teknologi, dan Kewirausahaan*, 1(2), 127–133.
- lii, B. A. B., Jenis, A., & Penelitian, D. (2014). Poltekkes Kemenkes Yogyakarta. September 2019, 30–41.
- Juliyanti, F. (2016). Administration of 70% Ethanol Extract of Kemuning Leaves (*Murraya paniculata* (L.) Jack) on the Decrease of MDA Levels and Increase of Catalase Activity in Red Blood Cells of Male White Rats Exposed to Cigaret Smoke. *Revista CENIC. Ciencias Biológicas*, 152(3), 28.
- Layli, A. N., & Saraswati, I. A. P. (2021). The Effect of Soaking Duration of Single Clove Garlic (*Allium Sativum*) on Antioxidant Content and Preference (Hedonic Test). *Journal of Health Information*, 11(2), 522–531.
- Lestari, K. S. D., Putra, I. W. G. A. E., & Karmaya, I. N. M. (2015). Exposure to Cigaret Smoke in Pregnant Women at Home and Its Risk of Increasing the Incidence of Low

Proceeding books:

The 3rd International Conference and Innovation Exhibiti on Global Education (ICEGE)

- Birth Weight Infants in Gianyar Regency. *Arsip Kesehatan Masyarakat dan Pencegahan Penyakit*, 3(1), 11–15.
- Lestari, S. R. (2021). *Single Clove Garlic: Its Efficacy and Benefits Monograph* (P. M. Rifa'i & P. A. Gofur (eds.)). Malang State University.
- Maisarah, M., Chatri, M., & Advinda, L. (2023). Characteristics and Functions of Alkaloid Compounds as Antifungals in Plants. *Serambi Biologi Journal*, 8(2), 231–236.
- Maudia, S. (2018). The Ability of 70% Ethanol Extract of Salam Leaves (*Syzygium polyanthum* (Wight) Walp.) to Inhibit Defects in Pregnant Mice Exposed to Cigaret Smoke.
- Medika, V., Akbar, A., & Medan, U. (2016). The Effect of Beta Carotene Administration on the Percentage of Live Mouse (*Mus musculus*) Fetuses Exposed to Kretek Cigaret Smoke. *Biomedical Journal*, 11(3), 15–22.
- Misde Yola, Wibowo, A., & Thaurhesia, S. (2021). Activity and Safety Test of the Combination of Garlic Extract (*Alium sativum*.l) and Candlenut Extract (*Aleurites molucanna*) as a Rabbit Fur Fertilizer. *Borneo Journal of Pharmascientech*, 5(2), 44–54.
- Mohan, M. S. G., Ramakrishnan, T., Mani, V., & Achary, A. (2018). Efecto protector del polisacárido sulfatado crudo de *Turbinaria ornata* sobre la hepatotoxicidad inducida por isoniazida y rifampicina y el estrés oxidativo en el hígado, riñón y cerebro de ratas albinas suizas adultas. *Revista India de Bioquímica y Biofísica*, 55(4), 237–244.
- Monica, A. (2018). The Ability of 70% Ethanol Extract of Red Amaranth Leaves (*Amaranthus tricolor* L.) to Inhibit Defects in Pregnant White Mice Exposed to Cigaret Smoke.
- Mutmainah, S. (2016). Induction of Adventitious Shoots in Single Clove Garlic (*Allium sativum*) with the Addition of BAP and NAA In Vitro. *Revista CENIC. Ciencias Biológicas*, 152(3), 28.
- Nurhayati, E., Bu'ololo, I., & Ndururu, S. (2020). Counseling on the Dangers of Smoking at Bayangkara Medan High School. *Jurnal Mitra Keperawatan Dan Kebidanan Prima*, 2(1), 4–5.
- Nursyahrina, E. A., & Lestari, P. P. (2021). La importancia de consumir frutas que contienen antioxidantes para el sistema inmunológico del cuerpo durante una pandemia. *Proceedings ...*, 28–32.
- Octariani, S., Mayasari, D., & Ramadhan, A. M. (2021). Actas de las Conferencias de Farmacéuticas de Mulawarman. *Actas de las Conferencias de Farmacéuticas de Mulawarman*, abril de 2021, 135–138.
- Prameswari, A. (2016). The Effect of Ethanol Extract of Purwoceng (*Pimpinella alpina*) on Body Weight, Body Length, and Length of Femur Bone Calcification in Wistar Rat Fetuses. *Information System*, 2(Cdc), 1–4.
- Pudiarifanti, N., & Farizal, J. (2022). Phytochemical Screening and Antibacterial Activity of Single Clove Garlic Extract against *Staphylococcus aureus*. *Higea Pharmacy Journal*, 14(1), 66.
- Ramadhani, A. (2020). Antioxidant Activity Test of 70% Ethanol Extract of Johar Leaves (*Cassia siamea* Lamk) on MDA and SOD Levels in Male Rat Blood Cells Induced by CCL4. *NBER Working Papers*, 89.
- Romsiah, Putri, P. E., & Erjon. (2020). Comparison of antioxidant activity of garlic extract and single clove garlic in fresh and fermented forms using the DPPH method. *Jurnal Ilmiah Bukti Farmasi*, V(1), 45–50.
- Safitri, R. N., & Syahrul, F. (2015). The Risk of Exposure to Cigaret Smoke in Anemia During Pregnancy. *Jurnal Berkala Epidemiologi*, 3(3), 327. Sampurna Sampurna, & Siti Thomas Zulaikhah. (2022). Cigaret smoke impacts the levels of C-Reactive Protein (CRP). *Journal of Health Research Suara Forikes*, 13(1), 183–187.
- Sari, N. S. (2016). Antioxidant and Photoprotective Activity Test of Ethyl Acetate Fraction of Ethanol Extract from Red Dragon Fruit Peel (*Hylocereus polyrhizus*). Muhammadiyah University of Yogyakarta.
- Sinaga, R. N. (2014). Exercise and Free Radicals. *Journal of Sport Science and Fitness*, 7, 8.
- Siregar, J. A. R. (2023). Teratogenic Effects of Diethylene Glycol (DEG) Administration on the Morphology of White Mouse Fetuses (*Mus musculus* L.). 1–14.
- Sodik, M. A. (2018). M. Ali Sodik, M.A. In *Smoking and Its Dangers* (Issue 1).
- Sudjatini, M. P. (2017). The Effect of Processing Methods on the Antioxidant Activity of Garlic Extract (*Allium sativum* L.) Kating and Sinco Varieties.
- Tuslinah, L., Elkanawati, R. Y., & Dewi, R. (2023). THE EFFECT OF THE FERMENTATION

Proceeding books:

The 3rd International Conference and Innovation Exhibiti on Global Education (ICEGE)

PROCESS OF LONE GARLIC (*Allium sativum* L.) ON ANTIOXIDANT ACTIVITY
USING THE DPPH METHOD (1,1-DIPHENYL-2-PICRYLHYDRAZYL). *Journal of
Pharmacopolium*, 5(3),

Zulaikhah, S. T., Wibowo, J. W., & Wibowo, M. S. B. (2021). The Effect of Young Coconut
Water on Endogenous Antioxidant Levels Due to Cigaret Smoke Exposure in Male
Wistar Strain Rats Siti Thomas Zulaikhah. 12(6), 290–293