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# ANTIOXIDANT ACTIVITY AND CYTOTOXICITY TEST OF EXTRACT COMBINATION OF JAMBU BOL LEAF (Syzygium malaccense L.), MANILA SAPODILLA LEAF (Manilkara zapota L.) AGAINST HSC-3 CELL LINE

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**Abstract**

In the herbal plants of jambu bol (*Syzygium malaccense L.*) and manila sapodilla (*Manilkara zapota L.*), especially in the leaves of the plant, it contains very strong antioxidants. According to research by Zaen Devi Maulani and Ekayanti Meiliza, the antioxidant activity value of ethanol extract of jambu bol leaves (*Syzygium malaccense L.*) is 3.297 ± 2.595 ppm and for ethanol extract of manila sapodilla leaves (*Manilkara zapota L.*) is 8.2786 µg/mL. The antioxidant activity of a compound is said to have very strong antioxidants if the $IC_{50}$ value is less than 50 ppm, strong $IC_{50}$ if the $IC_{50}$ value ranges from 50-100 ppm, moderate if the $IC_{50}$ value ranges from 100-150 ppm, and weak if the $IC_{50}$ value ranges from 150-200 ppm. This study is an experimental laboratory by measuring the antioxidant activity value ($IC_{50}$) using different solvents in the combination of the two simplicia and the toxicity concentration of the combination of the two simplicia using the CCK-8 assay method. The samples used were tested for determination to determine and ensure that the materials used were the correct samples to be tested and made into extracts. Furthermore, the extraction of samples with solvents was concentrated with a rotary evaporator to separate the active substances and solvents. The results of this study indicate that there is no significant difference in the antioxidant activity of the combination of jambu bol leaf extract (*Syzygium malaccense L.*) and manila sapodilla leaf extract (*Manilkara zapota L.*) in the positive control (ascorbic acid). There is a significant difference in the cytotoxicity of jambu bol leaf extract (*Syzygium malaccense L.*) and manila sapodilla leaf extract (*Manilkara zapota L.*) in the positive control (DMSO) on the viability of HSC-3 cells. The combination of jambu bol leaf extract (*Syzygium malaccense L.*) and sapodilla leaf extract (*Manilkara zapota L.*) at a concentration of 5-160 µg/mL did not show any cytotoxic effect on the viability of HSC-3 cells.

**Keywords:** Antioxidants, Cytotoxicity, *Syzygium malaccense L.*, *Manilkara zapota L.*

## Introduction

Cancer is one type of disease that is of particular and serious concern in the field of medicine, because it occurs in developing countries such as Indonesia and is the second largest cause of death after cardiovascular disease. Oral cancer is one of the cancers that should be watched out for. Citing data from


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the World Health Organization (WHO) in 2020, the number of cases of lip and oral cancer in the world reached more than 377 thousand with the number of deaths caused reaching more than 177 thousand people. Oral cancer cases in Indonesia according to GLOBOCAN (Global Cancer Observatory) data in 2020, there were 5.780 cases and as many as 3.087 of them died.

There are several factors that can affect the presence of oral cancer, such as lack of oral hygiene, smoking, consuming alcohol and using betel nut. In addition, there are also several factors that can affect oral cancer, including age, gender, and immunologic nutrition (Wibowo et al., 2022). One of the oral cancer cells is Squamous Cells. Squamous Cells are common in the oral cavity due to lack of early examination so that it becomes a serious problem in the oral cavity. HSC-3 cells are cancer cells that are widely used in research because they can show similarities with the original tumor (Ribeiro et al., 2018).

In addition to the above factors, there are other factors that can cause cancer, one of which is reactive oxygen species (ROS). ROS are compounds that contain reactive oxygen. Low levels of ROS have the function of activating and modulating signal transduction pathways, modulating the activity of redox-sensitive transcription factors and regulating cell defense. Conversely, high levels of ROS are toxic to cells and induce oxidative stress. Cancer cell resistance is associated with high antioxidant enzymes that counteract ROS in cancer cells (Hikmah, Febrial., Hardiany, Novi Silvia., 2017).

Treatment of cancer cells in the oral cavity other than chemotherapy and chemical treatment can use natural chemical compounds contained in plants. Herbal plants are active compounds that can be used as another treatment in cancer treatment because herbal plants have the least side effects of treatment compared to other treatments. Anticancer contained in herbal plants can be in the form of plant extracts or single active compounds isolated from a plant. Some plants that have many benefits are jambu bol (*Syzygium malaccense L.*) and manila sapodilla *(Manilkara zapota L.*).

Based on research, it is known that the pharmacological activity of jambu bol leaf extract (*Syzygium malaccense L.*) and manila sapodilla leaf extract (*Manilkara zapota L.*) as anti-inflammatory, anticancer, and antibiotic. Therefore, this research was conducted to determine the effect of the ethanol fraction of jambu bol leaf extract (*Syzygium malacense L.*) and manila sapodilla leaf extract (*Manilkara zapota L.)* on the cytotoxicity of HSC-3 cells.

In herbal plants jambu bol (*Syzygium malaccense L.*) and manila sapodilla (*Manilkara zapota L.*), especially in the leaves of these plants contain very strong antioxidants. According to research by Zaen Devi Maulani and Ekayanti Meiliza, the antioxidant activity value of ethanol extract of jambu bol leaf is 3.297 ± 2.595 ppm and for ethanol extract of manila sapodilla leaf (*Manilkara zapota L.*) is 8.2786 µg/mL. The antioxidant activity of a compound is said to have a very strong antioxidant if the $IC_{50}$ value is less than 50 ppm, strong $IC_{50}$ if the $IC_{50}$ value ranges from 50-100 ppm, moderate if the $IC_{50}$ value ranges from 100-150 ppm, and weak if the $IC_{50}$ value ranges from 150-200 ppm (Moniung et al., 2022).

**Method**

The type of research used is laboratory experimental by measuring the value of antioxidant activity ($IC_{50}$) using different solvents in the combination of the two simplicia and the toxicity concentration of the combination of the two simplicia using the CCK-8 assay method. The samples used were subjected to a determination test to determine and ensure that the materials used were true samples to be tested and extracted. Furthermore, extraction of samples with solvents, concentrated with a rotary evaporator to separate active substances and solvents. This study uses statistical analysis by conducting a normality test, one-way ANOVA test with Tukey's HSD post hoc analysis. The statistical significance of the data was

calculated by one-way analysis of variance (ANOVA) along with Tukey's HSD post hoc test to determine the level of significance. Results were considered significant if $p<0.005$.

**Results**

a. Antioxidant Test

Testing of antioxidant activity using the DPPH test is carried out at the maximum wavelength. Testing with DPPH can be seen and analyzed on changes in DPPH solution from concentrated purple to yellow color that occurs if the sample has antioxidant compounds (Baliyan et al, 2022; Fajriari and Jamaludin S, 2023). Strong or weak antioxidant compounds contained in the test sample can be seen in the %inhibition results obtained. The test results on the inhibition data can be seen in Table 4.1. The test results show a linear ratio between concentration and %inhibition, while the absorbance is inversely proportional.

**Table 1. Inhibition data on the extract combination of jambu bol leaf (*Syzygium malaccense L.*) and manila sapodilla (*Manilkara zapota L.*) leaf.**

| Sample | Concentration | Absorbance | | | Average Absorbance | %Inhibition |
|---|---|---|---|---|---|---|
| | | Data 1 | Data 2 | Data 3 | | |
| A | 0 | 0,799 | 0,796 | 0,799 | 0,798 | - |
| | 5 | 0,615 | 0,411 | 0,55 | 0,525 | 34,187 |
| | 10 | 0,385 | 0,331 | 0,311 | 0,342 | 57,103 |
| | 15 | 0,225 | 0,191 | 0,172 | 0,196 | 75,439 |
| | 20 | 0,113 | 0,1 | 0,1 | 0,104 | 86,926 |
| | 25 | 0,083 | 0,08 | 0,079 | 0,081 | 89,891 |
| B | 0 | 0,959 | 0,91 | 0,952 | 0,940 | - |
| | 1 | 0,799 | 0,797 | 0,787 | 0,794 | 15,478 |
| | 2 | 0,683 | 0,687 | 0,7 | 0,690 | 26,585 |
| | 3 | 0,593 | 0,556 | 0,595 | 0,581 | 38,189 |
| | 4 | 0,459 | 0,438 | 0,464 | 0,454 | 51,755 |
| | 5 | 0,344 | 0,317 | 0,333 | 0,331 | 64,772 |

Description: A= extract combination of jambu bol (*Syzygium malaccense L.*) and manila sapodilla (*Manilkara zapota L.*) leaf; B= Ascorbic Acid

The results of the sample testing on the variation of %inhibition obtained were made in the form of a curve to obtain the antioxidant profile of the sample. The curve consists of sample concentration and %inhibition data. The concentration of the samples varied as the constituent data on the curve on the X-axis, while the %inhibition as the constituent data on the curve on the Y-axis. The results of the antioxidant profile made a correlation relationship between the concentration and %inhibition values presented in Figure 1.

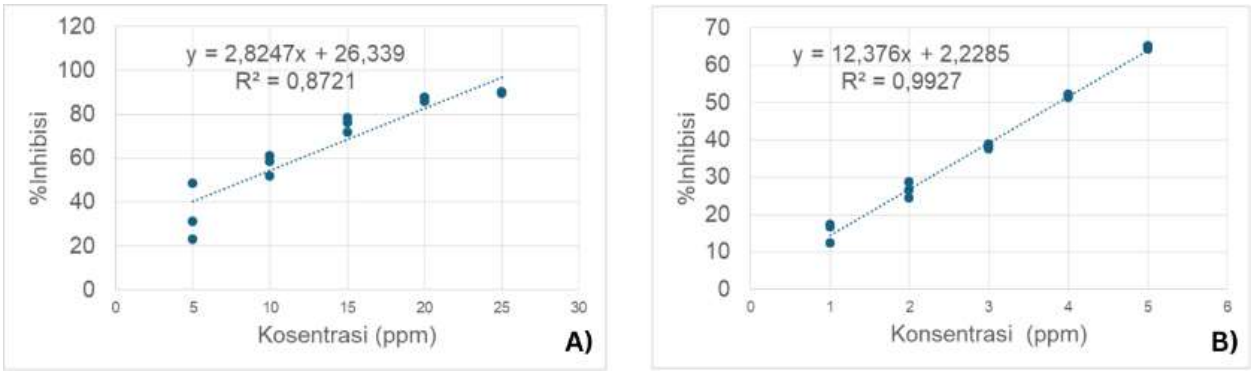


**Figure 1. Correlation curve of concentration with % inhibition of DPPH assay in (A) extract combination of jambu bol leaf (*Syzygium malaccense L.*) and manila sapodilla leaf (*Manilkara zapota L.*) (B) Ascorbic acid.**

The correlation between concentration and %inhibition results in a linear regression equation with the general equation y = bx + a which is used in determining the $IC_{50}$ value. The regression equation can be seen in Table 4.2 along with the $r^2$ value, $IC_{50}$ and standard deviation obtained.

**Table 2. $IC_{50}$ results data on the test sample**

| Sample | Regression Equations | $r^2$ | $IC_{50}$ (µg/mL) | Standard deviation |
|---|---|---|---|---|
| Extract Combination of Jambu Bol Leaf and Manila Sapodilla Leaf | $Y = 26{,}339 + 2{,}825$ | 0,872 | 8,01 | 2,76 |
| Ascorbic Acid | $Y = 2{,}228 + 12{,}376$ | 0,993 | 3,86 | 0,02 |

The closer the coefficient of determination ($r^2$) value is to 1, the more linear the correlation between concentration and %inhibition obtained. The $IC_{50}$ results obtained show antioxidant activity is classified as very strong which is characterized by $IC_{50}$ values <50 µg/mL in both samples. According to Moniung et al (2022) compounds with antioxidant activity are included in the very strong category if they have an $IC_{50}$ value <50 µg/mL, including the strong category if the $IC_{50}$ value is in the range of 50-100 µg/mL, including the medium category if the $IC_{50}$ value is in the range of 100-150 µg/mL, and including the weak category if the $IC_{50}$ value is in the range of 150-200 µg/mL.

**b. Cytotoxicity Test**

The data that has been obtained is analyzed by ANOVA to determine the significance of differences in means between test treatment groups. The difference in means can be said to be significantly different or significantly different if the p-value obtained is <0.05. The results of ANOVA statistical analysis can be seen in Table 4.7. The obtained p-value of 0.000 (<0.05) indicates that there is a difference in the mean levels of cytotoxicity that is significantly different so it is necessary to do the HSD Tuckey post hoc test.

**Table 3 The results of ANOVA data analysis on toxicity test results**

| Group | N | Mean | Std. Deviation | Say. |
|---|---|---|---|---|
| 160 µg/mL (2:1) | 3 | 1,26 | 0,14 | |
| 80 µg/mL (2:1) | 3 | 1,12 | 0,02 | |
| 40 µg/mL (2:1) | 3 | 1,01 | 0,10 | |
| 20 µg/mL (2:1) | 3 | 1,10 | 0,07 | |
| 10 µg/mL (2:1) | 3 | 0,98 | 0,07 | |
| 5 µg/mL (2:1) | 3 | 0,88 | 0,03 | |
| 160 µg/mL (1) | 3 | 1,19 | 0,06 | |
| 80 µg/mL (1) | 3 | 1,04 | 0,01 | 0,000 |
| 40 µg/mL (1) | 3 | 1,01 | 0,02 | |
| 20 µg/mL (1) | 3 | 1,17 | 0,03 | |
| 10 µg/mL (1) | 3 | 1,05 | 0,06 | |
| 5 µg/mL (1) | 3 | 0,82 | 0,06 | |
| No Treatment | 3 | 1,18 | 0,04 | |
| Solvent Control | 3 | 1,17 | 0,07 | |
| DMSO 10% | 3 | 0,03 | 0,00 | |

**Discussion**

1. **Antioxidant Activity of extract Combination of Jambu Bol Leaf (Syzygium malaccense L.) and Manila Sapodilla Leaf (Manilkara zapota L.)**

Antioxidant activity can be tested with DPPH using UV-Vis spectrophotometer. DPPH functions as a free hydrogen/radical giver. The principle of antioxidant test with DPPH is the decolorization to yellow which was originally dark purple due to the reduction of DPPH by antioxidant compounds (Baliyan et al, 2022). The stronger antioxidant compounds have the activity to inhibit DPPH (radical) more strongly so that the color of the DPPH solution becomes clearer/pale visually (Fajriani and Jamaludin S, 2023).

The results of the study regarding the level of inhibition of free radicals can be seen in Table 4.1. The sample used is a extract combination of jambu bol leaf (*Syzygium malaccense L.*) and manila sapodilla leaf (*Manilkara zapota L.*) and ascorbic acid. Ascorbic acid is used as a positive control and comparison of antioxidant activity contained in the extract combination of jambu bol leaf and sawo leaf. The higher % inhibition indicates the more antioxidant compounds contained in the sample. The results presented in Table 4.1 show the %inhibition in both samples showed the higher the concentration, the higher the %inhibition obtained, while the absorbance value is smaller. The smaller absorbance shows that the DPPH solution becomes clearer/brighter. The smaller absorbance is due to an increase in concentration / the presence of more antioxidant compounds in counteracting the number of free radicals / DPPH ((Fajriani, 2023), (Baliyan et al., 2022)). The relationship between concentration and %inhibition is presented in Figure 4.1. In Figure 4.1 both samples showed that the higher the concentration the greater the %inhibition. Both samples indicated a strong linear correlation between concentration and %inhibition. Linearity can be seen in the coefficient of determination ($r^2$) value. The $r^2$ value for the combination of sawo manila leaf and jambu bol leaf extracts was 0.872 and for ascorbic acid was 0.993. The coefficient of determination value >0.7 indicates a strong linear relationship (Rusakov, 2023).

Antioxidant compounds can be quantified based on the $IC_{50}$ value. $IC_{50}$ is a parameter that interprets the ability of antioxidants to reduce the concentration of DPPH by 50%. The smaller the $IC_{50}$ value, the

stronger the antioxidant activity. The $IC_{50}$ values of all tested samples have $IC_{50}$ values <50 which indicates very strong antioxidant activity. $IC_{50}$ result data on the test samples can be seen in Table 4.2. The extract combination of jambu bol leaf and manila sapodilla leaf had an $IC_{50}$ value of 8.01 µg/mL. The positive control, ascorbic acid, has an $IC_{50}$ of 3.86. The sample concentration ratio of the extract combination of jambu bol leaf and manila sapodilla leaf (5): (1) ascorbic acid as a positive control. This shows that ascorbic acid (positive control) has a stronger antioxidant activity compared to the antioxidant activity of the extract combination of jambu bol leaf and manila sapodilla leaf. In the research of Devitria et al (2023), the $IC_{50}$ value of ethanol extract of jambu bol is 3,511 µg/mL with the highest sample concentration variation of jambu bol single extract reaching 1000 µg/mL. As for (Alyidrus et al., 2021) regarding the antioxidant activity test of ethanol extract of manila sapodilla leaves singly (not combined) with the DPPH method, the $IC_{50}$ value obtained was 8.2786 µg/mL with a concentration variation of 20; 40; 60; and 80 µg/mL. In the research of Devitria et al (2023), the $IC_{50}$ value of ethanol extract of jambu bol is 3,511 µg/mL with the highest sample concentration variation of jambu bol leaf single extract reaching 1000 µg/mL. As for (Alyidrus et al., 2021) regarding the antioxidant activity test of ethanol extract of manila sapodilla leaf singly (not combined) with the DPPH method, the $IC_{50}$ value obtained was 8.2786 µg/mL with a concentration variation of 20; 40; 60; and 80 µg/mL. The difference in results is caused by differences in sample concentration, single or combined samples, growing location, harvest time, different solvents, or the presence of residues in the sample (Utomo et al., 2020).

The data obtained were statistically analyzed using normality tests with the Shapiro Wilk test. Based on the results of statistical analysis presented in Table 4.3, the p value>0.05 was obtained for the extract combination of manila sapodilla leaf and jambu bol leaf (0.898) and ascorbic acid (0.637). The results obtained indicate that the data obtained are normally distributed. Data that show normally distributed data can be analyzed to determine the significance of the difference in the mean of two samples using the independent T test. The results of the analysis are presented in Table 4.4. The results obtained were 0.06 (p>0.05) indicating there was no significant difference in the $IC_{50}$ value of the test sample of the extract combination of jambu bol leaf and manila sapodilla leaf and ascorbic acid as a positive control. The results of the analysis with a value of p>0.05 then no further statistical tests are needed.

**2. Cytotoxicity of Combination of Jambu Bol Leaf Extract (Syzygium malaccense L.) and Sawo Manila Leaf (Manilkara zapota L.)**

In the cytotoxicity test, a series of processes need to be carried out and begins with the preparation of the culture. HSC-3 cell culture grown on media is given antibiotics (penicillin and streptomycin) with the aim of preventing microbial contamination such as bacteria in cell culture. HSC-3 cell results that have been cultured and before and after treatment. In addition to the positive control (DMSO), the results showed HSC-3 cells in pre-treatment, post without treatment, and post with solvent control showed no significant difference, the cells were still alive and did not experience significant damage. The expected result is that cells began to experience significant damage shown only in the positive control, namely DMSO. Cells begin to experience stress or damage in the form of apoptosis or necrosis which is characterized by cells that experience fragmentation (Fristiohady, A., & Agustina, 2020).

The cytotoxicity test in this study used HSC-3 cells with the aim that the results of the study could interpret oral squamous cell carcinoma. This type of oral cancer occurs in the oral cavity area and is strongly influenced by its anatomical presence. The use of HSC-3 cells is often used in research because it belongs to tongue cancer cells and the results of previous studies using this type of cell can interpret results similar to the original tumor (Pakpahan, A., & Anggraeni, R, 2023). The cytotoxicity test method used is the

Counting Kit-8 (CCK-8 or WST-8) method. The basic principle of the CCK-8 method is the activity of the tetrazolium salt reduction reaction caused by cells through dehydrogenase in it so that formazan formation occurs which correlates with the quantity of living cells. This method has advantages in sensitivity when compared to methods such as MTT (Karim B.K and Hendriani, 2023).

The results of the cytotoxicity test are presented in Table 4.5. The results show that the average absorbance between the test treatments that have been given jambu bol leaf extract or a extract combination of jambu bol leaf and manila sapodilla with the treatment without being given the extract (solvent control) has an absorbance that is not much different so that the % of dead cells calculated based on the absorbance data obtained shows relatively very low results and even none. The % dead cells that are too low or relatively absent in extract samples with a concentration variation of 5 - 160 µg/mL can be influenced by concentrations that are too low to cause cytotoxic effects. In this study, the analysis could not be continued with probit numbers because this probit number is one of the data that correlates the level of probability with the results studied based on the response of live or dead cells that are converted by the system into probit scores. Only the positive control, 10% DMSO, showed a high % dead cells characterized by much lower absorbance data (mean absorbance 0.026) compared to the solvent control (mean absorbance 1.168). This indicates a higher % of dead cells. The concentration variation of 5 - 160 µg/mL in guava bol leaf extract and the extract combination of jambu bol and manila sapodilla used were too low to make the cells die so they did not reach $IC_{50}$. (Pakpahan & Anggraeni, 2023) reported that the concentration variation of 160-640 µg/mL of ethanol fraction extract was able to have a cytotoxic effect. The cytotoxic effect is directly proportional to the concentration, the higher the concentration, the higher the ability to kill cells.

The results of the cytotoxicity test obtained were analyzed statistically in the form of normality and ANOVA tests. In the normality test with Shapiro Wilk (Table 4.6) the results show the cytotoxicity data obtained p-value (0.000) <0.05 so that the data can be categorized as data that is not normally distributed. The results of the extract combination of jambu bol and manila sapodilla also showed results that were included in the category of data not normally distributed with a p-value (0.037) <0.05. The results of statistical analysis on ascorbic acid obtained a p-value (0.242) > 0.05 which indicates normally distributed data. In the ANOVA test, the p-value obtained was 0.000 (<0.05) indicating that there were differences in the mean levels of cytotoxicity that were significantly different so it was necessary to do Tukey's HSD post hoc test. Tukey's HSD analysis can be seen in Table 4.8. The results show that some group pairs have significant differences in mean cytotoxicity and some are not significant.

**Conclusion**

Based on the research that has been done, it can be concluded that:

1. There is no significant difference in antioxidant activity of the extract combination of jambu bol leaf (*Syzygium malaccense L.*) and manila sapodilla leaf (Manilkara zapota L.) on positive control (ascorbic acid).
2. There is a significant difference in the cytotoxicity test of the extract combination of jambu bol leaf (*Syzygium malaccense L.*) and manila sapodilla leaf (Manilkara zapota L.) on positive control (DMSO) on HSC-3 cell viability. The extract combination of jambu bol leaf and sawo manila leaf at concentrations of 5-160 µg/mL showed no cytotoxic effect on HSC-3 cell viability.

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