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Research Article

THE COMBINATION OF PROPOLIS AND *Curcuma zanthorrhiza* AS ANTI-BREAST CANCER MATERIALS

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ABSTRACT

Breast cancer is the second leading cause of death after cervical cancer from the many deaths caused by cancer in women. Cancer treatment therapy is generally classified as expensive and has serious side effects. Propolis and Curcuma zanthorrhiza (Curcuma zanthorrhiza) have been widely tested by research institutions and have the potential to be anticancer. This study aimed to evaluate the synergy between anticancer activity of a combination of propolis and Curcuma zanthorrhiza extract against MCF-7 cancer cells using Response Surface Methodology (RSM). The test parameters observed were % inhibition of combination of propolis and Curcuma zanthorrhiza extract against MCF-7 cancer cells. Tests were carried out with 13 treatment combinations of extracts of propolis and Curcuma zanthorrhiza, negative control (cell growth media MCF-7) and positive control (doxorubicin) carried out in 3 replications in each treatment. The results showed that the LC50 and IC50 values obtained were in a higher concentration range compared to the LC50 and IC50 values of each extract.

Keywords: breast cancer, MCF-7, propolis, Curcuma zanthorrhiza

INTRODUCTION

Cancer is a condition where the cell has lost its normal control and mechanism, thus experiencing rapid and uncontrolled growth, and is one of the leading causes of death in the world. The latest data from the 2013 published by [1] states that cancer prevalence reaches 0.14% and the estimated number of sufferers will increase to 374.792 residents. Based on GLOBOCAN data, breast cancer is the highest percentage of new cases in women by 43.1% with a percentage of deaths of 12.9% [2].

According to [3] breast cancer therapy is classified as surgery, radiotherapy, chemotherapy and hormonal therapy. These therapies often cause side effects such as the spread of cancer cells in other tissues, damage healthy cells, and can cause cancer cells to mutate until it is difficult to destroy. The discovery of new drugs that are effective, relatively safe and do not cause side effects are needed as an alternative treatment. Various types of medicinal plants have been tested as anticancer by research institutions, universities, and pharmaceutical companies, including propolis and *Curcuma zanthorrhiza* Roxb. (*C. xanthorrhiza*)

Propolis can attack cancer [4]. Researchers [5] and [6] states that propolis from Taiwan and Brazil has cytotoxic components and has the potential to be anticancer. Researcher [7] concluded that ethylacetate propolis extract has cytotoxic activity and triggers apoptosis in MCF-7 cancer cells. Researcher [8] states that nanopropolis at a concentration of 32 µg / mL equivalent to propolis at a concentration of 233 µg / mL can reduce tumor cell size and can be used in the treatment of breast cancer and tumors in mammary mice cells.

Curcuma zanthorrhiza is a finding that belongs to the *Zingiberaceae* family. The part of the *Curcuma zanthorrhiza* plant that is used is the rizhome. *Curcuma zanthorrhiza* rhizome contains the main active components which are efficacious, namely curcuminoids (curcumin, bismetoxicurumum and demetoxycurcumin) and essential oils (feladren, kamfer, tumerol, methocarbamol, ar-curcumen, zingiberen, kuzerenon, germacron, β-tumeron, and xanthoryzole) [9]. Several studies have shown that *Curcuma zanthorrhiza* has the potential as an anticancer. Xantorizole in *Curcuma zanthorrhiza* has the potential as a chemopreventive and anticancer agent by showing antiproliferation activity against MCF-7 cells [10]. The combination of xanthorizole and curcumin has the potential to antiproliferate against breast cancer cells [11].

The Brine Shrimp Lethality Test (BSLT) method is used for the initial screening test of the anticancer activity of propolis and *Curcuma zanthorrhiza* extract using *Artemia salina* Leach. This method is used as a guided fractionation bioassay from natural materials because it is easy, fast, and inexpensive and can be used to predict the toxicity of test samples [12]. The results of the toxicity test were known from the number of deaths of *Artemia salina* due to the effect of extract at a predetermined dose. The data is analyzed to determine the LC_{50} value. The extract tested was declared to have anticancer activity if the LC_{50} value of each extract was less than 1000 µg / mL [13]. Researcher [14] stated that the LC_{50} value of *Curcuma zanthorrhiza* ethanol extract was 238 µg / mL, while the LC_{50} value of propolis was reported in the [15] study (2016) which was 16,010 µg / ml. Researcher [7] stated that the IC_{50} value of ethylacetate propolis extract was 47.45 µg / mL, while the IC_{50} value of curcumin was reported in the study of [16] that is 35 µg / mL.

This study aims to evaluate the synergicity of propolis and *Curcuma zanthorrhiza* extracts against MCF-7 cancer cells using Response Surface Methodology (RSM), aimed at obtaining response optimization [17]. Data analysis was performed with

RSM after testing the cytotoxic activity of propolis and *Curcuma zanthorrhiza in-vitro* against MCF-7 cancer cells.

MATERIALS AND METHODS

Rhizome of *Curcuma zanthorrhiza* (from West Java, Indonesia) and raw propolis (from Central Java, Indonesia), cancer cells MCF-7 from the collection of Agency for the Assessment and Application of Technology, Indonesia.

Propolis Extract

Propolis is extracted by maceration using a modified method from [19] and [20]. Extraction using a combination of maceration method using 70% ethanol solvent and microwave heating (Microwave-assisted extraction, MAE). A total of 40 g of raw propolis was heated with a microwave heater for 5 minutes, then macerated with 360 mL of 70% ethanol and shaken with an orbital shaker for 24 hours at a speed of 120 rpm. The extract is filtered and the filtrate obtained is poured on a steam plate and heated at 50ºC until the ethanol evaporates. The extract yield was obtained by calculating the weight percent of the extract against the weight of extracted raw propolis (equation 1).

% Yield = (extract weight)/(simplicia weight) x 100%.

Curcuma zanthorrhiza Extract

Curcuma zanthorrhiza extracted by maceration refers to the research of [14]. *Curcuma zanthorrhiza* powder as much as 100 g was extracted by maceration method using 70% ethanol solvent with a ratio of simplified powder: solvent (1:6) for 3 hours at 40-45ºC. The extraction results are concentrated using a steam cup above the waterbath. The yield was obtained by calculating the weight percent of the extract against the simplicia weight (equation 2).

% Yield = (extract weight)/(simplicia weight) x 100%.

Phytochemical Test

Phytochemical test was carried out on propolis extract, and *Curcuma zanthorrhiza* extract [21].

Alkaloid Test

A weighted sample of 0.5 g was then added with 1 mL of 2N hydrochloric acid and 9 mL of water, heated on a water bath for ± 15 minutes, cooled and filtered. The solution was dripped on the watch glass, and each Dragendorff, Mayer and Bouchard reagent was added. The color of the precipitate formed is recorded [22].

Flavonoid Test

The test solution is made by weighing as much as 2 g of the sample and is treated with 10 mL of methanol, heated for 10 minutes and filtered. The filtrate is evaporated and diluted with 10 mL of water, then cooled. Solution added 5 ml of petroleum ether, shake carefully, let stand, then take a methanol-water layer and evaporate. The residue is dissolved in 5 mL ethyl acetate, and filtered.

The test solution was evaporated to dryness, adding 2-3 drops of ethanol, then added with Mg powder and a few drops of 5M hydrochloric acid. The red to purple violet that arises indicates the presence of flavanone, flavonol, flavone and dihydroflavonol. The same test is done using Zn powder, red to violet red indicates the presence of dihydroflavonol compounds, while flavanone and flavonoids are colorless or weak pink [22].

Tannin Test

The sample was weighed as much as 2 g then extracted with 80% ethanol (30 mL) for 15 minutes and filtered. The filtrate obtained is evaporated above the bath. Hot aquadest are added to the remaining evaporation, then stirred, cooled and then centrifuged. The top liquid is separated by decantation, and the solution is used as an experimental solution which will be used in the following test [22]:

a. The filtrate is added with a solution of 10% gelatin, white precipitate will appear.
b. The filtrate was added to NaCl-gelatin (1% solution of gelatin in 10% NaCl with a ratio of 1:1). Deposits arise and are compared with the results in part a.
c. The filtrate is added with a solution of 3% $FeCl_3$, occurring in blue to blackish green.

Saponin Test

A 0.5 g sample is put into a test tube, added 10 ml of hot water, cooled and then shaken vigorously for 10 seconds. Positive reaction if it forms a solid foam for not less than 10 minutes, as high as 1 cm to 10 cm, in addition 1 drops of 2 N hydrochloric acid are not lost [22].

Steroids Test

The sample was weighed as much as 0.5 g and dissolved with ethanol, then added 2 mL of anhydrous acetic acid and 2 mL of concentrated H_2SO_4. Changing the color of violet (purple) to green indicates the presence of steroids [23].

Terpenoids Test

The sample was weighed as much as 0.5 g and dissolved with 2 mL chloroform, then added 3 mL concentrated H_2SO_4 drop by drop until a layer formed. The sample is said to contain terpenoids if reddish brown is formed at the boundary between layers [23].

Toxicity Test of Propolis and *Curcuma zanthorrhiza* Extract BSLT Method

Hatching of *Artemia salina* eggs is carried out on clear containers such as beakers or jars that are given plastic material, film negatives, or glass using salt water with a salt content (NaCl) of 15 g / L. The hatching temperature is maintained at 25-30ºC using a 40-60 watt incandescent / neon lamp for 48 hours. The oxygen level needed during hatching must be more than 3 mg / L, so that it is given air using an aerator, compressor or blower. Active 48-hour *Nauplii* is used as a test animal in the study [12].

Stock solution is made by means of 70% ethanol extract of *Curcuma zanthorrhiza* and propolis weighed as much as 100 mg and diluted with salt water in a 50 mL volumetric flask to the boundary markings. The parent solution is pipetted as much as 8; 4; 2; 1; 0.5 and 0.25 mL and put into each vial that has been held for 10 mL so that the concentrations of 150, 125, 100, 75, 50 and 25 µg / mL vials were obtained. Negative control (blank) only contains salt water without the addition of extract. *Artemia salina* was inserted as many as 10 animals into each vial and added salt water to the mark of the tera border. One drop of yeast suspension (0.6 mg / mL) was added to each vial as food for shrimp larvae. Tests were carried out as many as 3 repetitions and observations were made for 24 hours [12]. Value which is made by the line equation 3.

% death = (total death-total death control)/(total start larvae (10)) x 100%.

The data obtained were analyzed using Probit Analysis. The LC_{50} value is determined based on the correlation curve between the extract concentration log (x-axis) and the probit (y-axis) value which is made by the line equation 4.

Y = a+bx; with y = probit value and x = extracts concentration.

Toxicity Test for Combination of Propolis and *Curcuma zanthorrhiza* BSLT Method

Toxicity testing of a combination of propolis and *Curcuma zanthorrhiza* extract was continued after obtaining the LC_{50} values of each extract from the results of the previous test. The comparison of the concentration used is obtained from the results of running RSM inputted based on the LC_{50} value of each extract as a center point. This test was carried out in various treatments to determine the model of the relationship of factor variables to the response, there were 2 independent variables that were considered as variables affecting% of *Artemia salina* mortality as a response, namely the concentration of *Curcuma zanthorrhiza* (X_1) and the concentration of propolis (X_2).

Cytotoxic Test of Methyl Thiazol Tetrazolium (MTT) Assay against MCF-7 Cells

Propolis and *Curcuma zanthorrhiza* extract were weighed 4 mg each and dissolved in 1000 µL of RPMI (Roswell Park Memorial Institute) 1640 media to obtain concentrations of 4000 µg / mL, then vortexed until homogeneous. The main solution was made by piping 250 µL of sample solution of 4000 µg / mL and diluted with RPMI 1640 media to obtain a concentration of 1000 µg / mL. Concentration series solutions for testing are made according to the results of running from RSM. RPMI 1640 media was used as a negative control and doxorubicin as a positive control.

MCF-7 cells that had been grown on the flask were subcultured in 96 well plates for 24 hours at 5% CO2 at 37ºC with a number of 5000 cells / well. The extract combination is put into the well according to the series of concentrations that have been determined and re-incubated for 48 hours. Cell media was discarded and cells at 96 well plates were rinsed with PBS (Phosphate Buffer Saline), then added MTT reagent (5 mg / mL) as much as 10 µL per well and incubated for 4 hours. The supernatant is removed and ethanol is added to lysis the cell membrane and dissolve formazan crystals. Reading of Optical Density (OD) was carried out using ELISA (enzyme linked immunosorbent assay) reader at a wavelength of 595 nm [24].

The absorbance data from ELISA readings were converted into% cell inhibition using the following formula (equation 5):

% inhibition = ((Absorbance control-Absorbance treatment)/(Absorbance control)) x 100%.

The relationship of percentage of inhibition and concentration was then analyzed using linear regression test, then the extract concentration was calculated to inhibit 50% cancer cell growth (IC_{50}) [24].

Experimental design

The experimental design used in this study was the Response Surface Methodology method which was carried out with a second order Central Composite Design (CCD) model (multiple quadratic regression models) to see the combined effect or combination of two independent variables, namely X_1 = the concentration of propolis extract and X_2 = concentration of curcuma extract, with the dependent variable (response) that is Y = the percentage of dead cells. Data analysis will be carried out using Design Expert 7.1.5 free trial.

Cytotoxic testing of a combination of propolis and *Curcuma zanthorrhiza* extract was carried out based on IC_{50} values from literature studies. Researcher [7] stated that the IC_{50} value of propolis was 47.45 µg / mL, while the IC_{50} value of *Curcuma zanthorrhiza*, according to [16] was 35 µg / mL. Comparison of test concentration is obtained from running RSM results with a center point ½ of the IC_{50} value of each extract. Tests were carried out in various treatments to determine the model of the relationship of factor variables to the response, there were 2 independent variables that were considered as variables that affect % inhibition of combination extracts against sustainable cells of MCF-7 cancer, namely the concentration of *Curcuma zanthorrhiza* (X_1) and the concentration of propolis (X_2).

Model Central Composite Design with Design Expert 7.1.5 (Free Trial).

The Central Composite Design model for cytotoxic testing of a combination of propolis and *Curcuma zanthorrhiza* on cancer-sustainable cells MCF-7 which was inputted in the design expert 7.1.5 free trial program can be seen in Table 1.

Table 1. Input level of two test variables

Treatment	units	Level				
		-α	-1	0	+1	+α
Curcuma zanthorrhiza extracts	µg/mL	6.895	10	17.5	25	37.865
Propolis extracts	µg/mL	9.585	13.725	23.745	33.725	37.865

RESULT AND DISCUSSION

Plant Determination

Curcuma zanthorrhiza used in this study was obtained from the Nagrak area, Sukabumi Regency, West Java, Indonesia and has been determined in the Herbarium Bogoriense, the Botany Field of Biology Research Center, Indonesian Institute of Sciences (LIPI) Bogor, specimen number:560/IPH.1.01/If.07/III/2018. The results of the determination stated that the curcuma used in this study was *Curcuma xanthorrhiza* Roxb. which belongs to the Zingiberaceae.

Results of the Water Content of Propolis and *Curcuma zanthorrhiza* Extract

Determination of the water content of propolis and *Curcuma zanthorrhiza* extract was carried out using the Gravimetric method in duplicate for each extract. The average yield of propolis extract water content is 9.72%. Terms of extract water content in general is <10% [25], then the propolis extract water content obtained meets the requirements. The average yield of *Curcuma zanthorrhiza* extract water content was 10.1858%. The requirements of the water content of *Curcuma zanthorrhiza* extract according to the [25] are <10%, so the moisture content of *Curcuma zanthorrhiza* extract obtained does not meet the requirements but is still close to the stipulated conditions.

Results of Ash Levels of Propolis and *Curcuma zanthorrhiza* Extract

Determination of ash content of propolis and *Curcuma zanthorrhiza* extract was carried out using the Gravimetric method in duplicate for each extract. The average yield of ash content of propolis extract is 2.2696%. Terms of extract ash content in general is not more than 4% [25], so that the ash content of propolis extract meets the requirements. The average yield of *Curcuma zanthorrhiza* extract ash content was 6.8233%. The ash content of *Curcuma zanthorrhiza* extract obtained fulfilled the requirements because it was stated in [25] that *Curcuma zanthorrhiza* extract <7.8%

Phytochemical Test Results of Propolis Extract, *Curcuma zanthorrhiza* Extract

Phytochemical tests were carried out on simplicia powder and Curcuma zanthorrhiza extract as well as on propolis extracts based on the [22] method (2015) and [23]. Phytochemical test results can be seen in Table 2.

Table 2. Phytochemical test results, *Curcuma zanthorrhiza* extract and propolis extract

Compound Group	*Curcuma zanthorrhiza* Extracts	Propolis Extracts
Flavonoids	+	+
Tannins	-	+
Alkaloids	-	-
Saponins	-	+
Terpenoids	+	-
Steroids	-	-

+ : contains a class of test compounds, -: does not contain a class of test compounds

In Table 2 it can be seen that alkaloid compounds were identified in *Curcuma zanthorrhiza*, but not identified in curcuma extract. Phytochemical test of alkaloid compounds in Mayer, Bouchard, and Dragendroff's reagents obtained positive results on Bouchard and Dragendroff's reagents only, while in Mayer reagents obtained negative results. The results of the negative alkaloid test on *Curcuma zanthorrhiza* extract can be caused by inaccuracy or error in concluding, because according to [27], the phytochemical test method for the alkaloid compounds used has disadvantages, that these reactants not only precipitate alkaloids but can also precipitate some types of compounds, including proteins, coumarin, α-python, hydroxy flavones, and tannins.

Phytochemical test results of propolis extract can be seen in Table 2 that the propolis extract tested contained tannins, saponins and flavonoids. The test results are in accordance with the results of the study of [28] which states that ethanol extract of propolis contains tannins, saponins and flavonoids for propolis obtained from the same area namely Kendal. Propolis extract obtained in solid form when it is at low temperatures and in the form of sticky thick paste at room temperature. Propolis extract in this study was blackish brown with a yield of 15.06%. The antioxidant test results of propolis extract had IC_{50} values of 262.39 ± 16.32 and the category was medium.

Toxicity Test Results of Propolis and *Curcuma zanthorrhiza* Extract with BSLT Method.

Ethanol extract 70% *Curcuma zanthorrhiza* and propolis were tested for toxicity using the Brine Shrimp Lethality Test (BSLT) method using brine shrimp (*Artemia salina* L.) as a test animal. The BSLT method is used as a guided fractionation bioassay from natural materials, because it is easy, fast, inexpensive, and shows a correlation to a specific anticancer test in several compounds [12]. Toxicity was determined by looking at the value of the Lethal Concentration 50 % (LC_{50}) which was known by counting the number of *Artemia salina* that died due to the effect of the extract and calculated based on probit analysis.

The main solution of propolis and *Curcuma zanthorrhiza* extract was made in 2000 µg / mL by dissolving 100 mg of each extract in 50 mL of salt water, then the preliminary test made extract concentrations of 150, 125, 100, 75, 50 and 25 µg. / mL. After 24 hours of exposure, the LC_{50} value of the test sample is determined based on the correlation curve between the log extract concentration (x-axis) and the probit (y-axis) value [13]. The data obtained were analyzed using Probit Analysis to determine LC_{50} by looking at each series of concentration of the number of shrimp deaths with a 95% confidence interval. Probit analysis is used to determine the LC_{50} value, if the LC_{50} value of the extract tested is less than 1000 µg / mL then it is declared efficacious as an anticancer [13]. Calculation of LC_{50} value based on the number of dead *Artemia salina* obtained the average value for propolis extract and *Curcuma zanthorrhiza* extract was 40.67 ug / ml and 45.08 ug / ml.

Judging from the test results, propolis and *Curcuma zanthorrhiza* extract has the potential as anticancer because both have LC_{50} less than 1000 µg / mL and are included in the toxic category according to [13]. The *Curcuma zanthorrhiza* ethanol extract tested on *Artemia salina* in this study produced an LC_{50} value of 45.08 µg / mL. These results differ greatly from the results of [14] which obtained LC_{50} of 238.23 µg / mL in extracts obtained by the same extraction method. LC_{50} of propolis extract on the results of [15] study was obtained at 16.010 µg / mL, whereas in this test the LC_{50} value was 40.67 µg / mL, this could be influenced by the state of the test and the different compounds in the extract used.

BSLT test combination of propolis and *Curcuma zanthorrhiza* extract was continued after obtaining LC_{50} values of each extract. Comparison of the concentration used is obtained from the results of running RSM. This test was carried out in various treatments to determine the model of the relationship of factor variables to the response, there were 2 independent variables that were considered as variables that affected% of *Artemia salina* deaths, namely the concentration of *Curcuma zanthorrhiza* (X_1) and the concentration of propolis (X_2).

Based on the RSM analysis obtained the equation $Y = 44.1184 - 0.2710X_1 - 0.8424X_2 - 6.30607E-0.16X_1X_2 + 0.0076X_1^2 + 0.0139X_2^2$ with $R^2 = 0.88$. The positive sign of the coefficient shows that the dependent variable (% inhibition) is influenced by the independent variable and vice versa, so that from the equation it can be seen that the effect of each independent variable and interaction between variables shows the effect of negative significance (no significant effect). ANOVA test data showed the p-value which is the significance of the influence of the variable on the response. The results of the p-value of *Curcuma zanthorrhiza* (0.0015) and propolis (0.0105) were obtained smaller than the number of significance set at 0.05, this indicates that the ratio of *Curcuma zanthorrhiza* and propolis significantly affected the response. The significance value of the combination of *Curcuma zanthorrhiza* and propolis obtained p-value greater than 0.05. The ANOVA table data analysis results also show that there is a suitability of the model based on the results of the Lack of Fit obtained (p-value> α) = 0.0737. The surface contour of BSLT test results of a combination of propolis and *Curcuma zanthorrhiza* extract on the% of *Artemia salina* mortality can be seen in Figure 1.

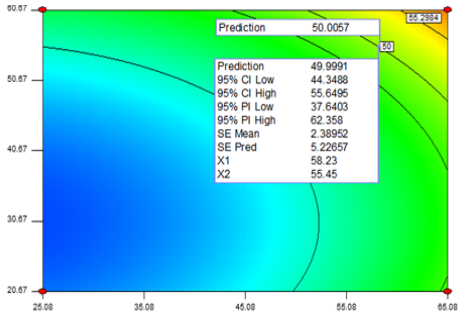

Figure 1. The surface contour of BSLT test results is a combination of propolis and *Curcuma zanthorrhiza* extract against of *Artemia salina* deaths

The predictive value of LC_{50} combination of *Curcuma zanthorrhiza* and propolis extracts based on Figure 1 was found in the concentration of *Curcuma zanthorrhiza* in the area of 58.23 µg / mL and the concentration of propolis in the area of 55.45 µg / mL. The LC_{50} value obtained is not much different from the single LC_{50} extract value that has been done before, which presents the LC_{50} value of *Curcuma zanthorrhiza* extract and propolis, respectively 45.08 and 40.67 µg / mL. These results indicate that the combination of *Curcuma zanthorrhiza* extract and propolis has a toxic effect on *Artemia salina*, but when compared to the results of the *Curcuma zanthorrhiza* and propolis extracts alone showed that the toxicity of the *Curcuma zanthorrhiza* and propolis extract decreased when combined, it can be seen from the LC_{50} value of the combination of both higher than the value LC_{50} extract of propolis and *Curcuma zanthorrhiza* the single extract test.

Cytotoxic Test Results of Propolis and *Curcuma zanthorrhiza* Extract with MTT Assay Method on MCF-7 Cancer Cell Culture

Cytotoxic testing of a combination of propolis and *Curcuma zanthorrhiza* extract was carried out based on IC_{50} values from literature studies. Researcher [7] stated that the IC_{50} value of propolis was 47.45 µg / mL, while the IC_{50} value of *Curcuma zanthorrhiza*, according to [16] was 35 µg / mL. Comparison of test concentration is obtained from running RSM results with a center point ½ of the IC_{50} value of each extract. Tests were carried out in various treatments to determine the model of the relationship of factor variables to the response, there were 2 independent variables that were considered as variables that affect% inhibition of combination extracts against sustainable cells of MCF-7 cancer, namely the concentration of Curcuma zanthorrhiza (X_1) and the concentration of propolis (X_2). Test results of various treatments are presented in Table 9 with a response in the form of % inhibition. ANOVA test data shows the p-value which is the significance of the influence of the variable on the response. All p-value values obtained are greater than the set of significance values of 0.05. This shows that the test variables namely propolis and *Curcuma zanthorrhiza* as well as a combination of both with the variation of the tested concentration had no significant effect on the response. The ANOVA table data analysis results also show that there is a suitability of the model based on the results of the Lack of Fit obtained (p-value> α) = 0.9648. The morphology of MCF-7 cancer cells before and after the treatment of a combination of propolis and *Curcuma zanthorrhiza* extract can be seen in Figure 2.

Based on the RSM analysis obtained the equation $Y = 21.10088 - 0.23809X_1 - 1.70285X_2 - 0.00461X_1X_2 + 0.025846X_1^2 + 0.037101X_2^2$ with $R^2 = 0.38$. The positive sign of the coefficient shows that the dependent variable (% inhibition) is influenced by the independent variable and vice versa, so that from the equation it can be seen that the effect of each independent variable and interaction between variables shows the effect of negative significance (no significant effect). R square value (R^2) obtained is relatively small and shows that the relationship between test variables is low. Analysis of variance in the mathematical equation model of the effect of the combination of curcuma and propolis extracts on the sustainable cell inhibition of cancer MCF-7. The surface contour of the MTT test results of the combination of propolis and *Curcuma zanthorrhiza* extracts on the inhibition of MCF-7 cancer cell culture can be seen in Figure 3.

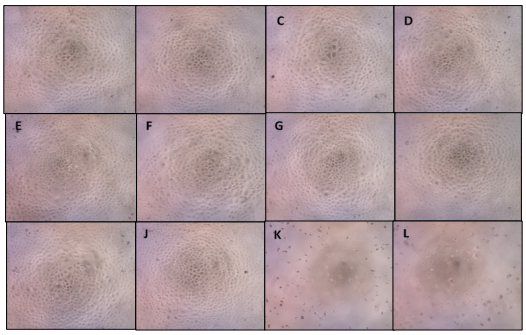

Figure 2. MCF-7 cancer cells result from a combination treatment of propolis and *Curcuma zanthorrhiza* extracts at 10 x. (A = control (-), B = unit of experiment 1, C = unit of experiment 2, D = unit of experiment 3, E = unit of experiment 4, F = unit of experiment 6, G = unit of experiment 7, H = unit of experiment 9, I = experimental unit 11, J = unit experiment 13, K = doxorubicin control (1 ppm), L = doxorubicin control (0.5 ppm))

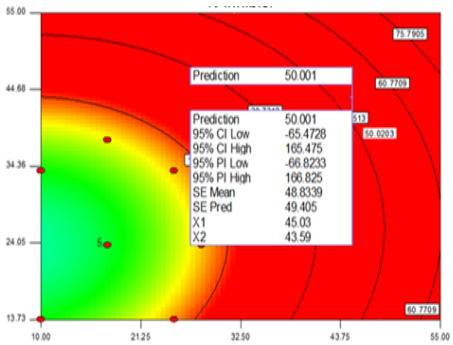

Figure 3. Surface contour of MTT test results of combination of propolis and *Curcuma zanthorrhiza* extract against inhibition of MCF-7 cancer cell culture

In the range of concentration of the test concentration was not obtained which gave of cell proliferation inhibition 50%, so that the IC_{50} value was obtained by looking at the predicted value of IC_{50} combination of *Curcuma zanthorrhiza* extract and propolis on the surface contour of the design expert 7.1.5 (free trial) software used. The predicted value of IC_{50} combination of *Curcuma zanthorrhiza* and propolis extract seen in Figure 3 is in the concentration of *Curcuma zanthorrhiza* in the area of 45.03 µg / mL and the concentration of propolis in the area of 43.59 µg / mL. The results obtained showed that the combination activity of *Curcuma zanthorrhiza* and propolis extract had a good enough effect on the inhibition of cancer cell growth, as seen from the % inhibition obtained in the combination of *Curcuma zanthorrhiza* and propolis extracts at test concentrations of 17.5 µg / mL and 23.73 µg / mL, respectively; which results in 17.148% inhibition of cell growth.

When viewed based on LC_{50} values from the results of the toxicity test of the BSLT method on *Artemia salina* and IC_{50} values from the cytotoxic assay of the MTT assay method on

MCF-7 cancer cells, a combination of propolis and *Curcuma zanthorrhiza* extract showed quite good activity. Predictive values for LC_{50} and IC_{50} each showed that the combination of propolis and *Curcuma zanthorrhiza* extract showed quite good activity, but the resulting effect was not much different from the results of the single extract test. The use of a combination of *Curcuma zanthorrhiza* extract and propolis is not recommended if viewed from an economic perspective if the use of *Curcuma zanthorrhiza* and propolis extracts alone is able to give the expected effect.

Many chemical compounds that can inhibit tumor cell growth, but not all compounds can trigger apoptosis. The main active components of propolis namely flavonoids and phenolic acids contained in propolis ethanol extract have many biological and pharmacological activities, one of which is the immune-potensive and antitumor effects by triggering apoptosis in MCF-7 cells [7]. Whereas *Curcuma zanthorrhiza* which has the main active components namely curcumin and xanthorizol with the mechanism of action inhibits cell proliferation (anti-proliferation) against breast cancer cells [16].

In previous studies propolis was said to be synergistic with chemotherapy agents, for example paclitaxel. Researcher [29] stated that the combination of paclitaxel and propolis was more effective in suppressing breast cancer growth when compared with the treatment of paclitaxel or propolis alone against mice induced by dimethyl benz-(a) anthracene (DMBA). In [30] also said that the combination of curcumin with mitomycin C (MMC) chemotherapy agents at a dose of 1.5 mg / body weight can synergistically inhibit tumor growth in MCF-7 breast cancer xenografts and induce apoptosis in MCF-7 breast cancer. Other studies have suggested that the combination of xanthorizol and curcumin showed synergistic activity inhibiting the growth of MDA-MB-231 breast cancer cells through apoptosis induction [11].

CONCLUSION

Lethal Concentration (LC_{50}) value of the results of the toxicity test of the BSLT method on *Artemia salina* and Inhibition Concentration (IC_{50}) values from the results of the MTT assay cytotoxic test on cancer cells MCF-7, a combination of propolis and *Curcuma zanthorrhiza* extract showed a fairly good activity. Predictive values for LC_{50} and IC_{50} each showed that the combination of propolis and *Curcuma zanthorrhiza* extract showed quite good activity, but the resulting effect was not much different from the results of the single extract test.

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