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EXTRACTION OF ROBUSTA COFFEE BEANS (*Coffea canephora*) FROM WONOSOBO BY ULTRASONIC WAVES AND ANTICANCER TESTS

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ABSTRACT

Wonosobo Robusta coffee beans are one of the natural source ingredients that potentially could be used as an anticancer. It contains polyphenolic compounds such as chlorogenic acid, flavonoids, and hydroxy hydroquinone. This research aims to determine the optimum conditions for the extraction temperature and time of the *Ultrasonic-Assisted Extraction* (UAE) method on the extract yield and to determine the anticancer activity. Extraction optimizations were analyzed using Response Surface Methodology (RSM) with Central Composite Design (CCD) experimental design on a temperature factor of 40, 50, 60 ºC and extraction times of 10, 30 and 50 minutes. Determination of anticancer activity was conducted using the *Methyl Thiazole Tetrazolium* (MTT) Assay method against MCF-7 cells. The optimization results indicate that the temperature of 52.74 ºC and time of 29.53 min are the optimum conditions for extraction with an extract yield of 9.16%. The anticancer activity test result at a concentration of 400 µg/mL is 83.25% so it has the potential to be used as an anticancer.

Keywords: *Coffea canephora, optimizations, UAE, anticancer MCF-7*

INTRODUCTION

Breast cancer occupies the highest position in Indonesia which is the main cause of death in Indonesian women. Based on data from the Global Burden of Cancer Study (Bray et al., 2018), there were 58,256 new cases of breast cancer with 22,692 deaths in Indonesia. Breast cancer is based on the first order of inpatients based on data from the Dharmais Cancer Hospital and increases every year due to changes in lifestyle (Ministry of Health of the Republic of Indonesia, 2015). Breast cancer is a tumor that spreads uncontrollably in the breast tissue consisting of the mammary glands, gland ducts, and breast supporting tissue which is the main cause of death in Indonesian women (Utami et al., 2018). Long-term breast cancer treatment using chemotherapeutic agents causes cancer cell resistance and toxic effects on normal body tissues. One source of natural ingredients that have the potential as anticancer are polyphenolic compounds such as chlorogenic acid (Gouthamchandra et al., 2017), flavonoids (Rao & Nadumane, 2016), and hydroxy hydroquinone contained in coffee beans (Shashni et al., 2022). Robusta coffee grows very well at an altitude of 400 - 700 above the sea level making Wonosobo,

which is dominated at an altitude of 500 - 1000 above the sea level, an ideal place for coffee cultivation. Based on research Utami et al. (2018) Wonosobo Robusta coffee beans have very active antioxidant activity with an IC_{50} value of 46.71 mg/L compared to other Robusta coffees from Central Java.

To extract polyphenolic compounds in Robusta coffee beans, ultrasonic wave assisted extraction (UAE) method was used (Sholihah, 2017). According to the research of Safdar et al. (2017) the polyphenolic content of mango peel extract extracted by the UAE method was 13.12% higher than the maceration method. To produce high-quality extracts, it is necessary to optimize the UAE extraction using Response Surface Methodology (RSM) so that it can consider the influence of two or more factors in one experiment and can estimate the interaction between the factors used to make it easier to identify the factors that have the most significant influence on the response (Fajrin & Marchelina, 2017). In the research of Syakfanaya et al. (2019) 30 min is the optimum time to extract chlorogenic acid and caffeine compounds from Robusta green coffee beans of 13.06 mg/g and 4.85 mg/g. Determination of antioxidant activity in tobacco leaves with a time factor of 15, 30, 45 min and temperature 30, 50, 70 ºC with ethanol solvent showed antioxidant activity with the highest percent inhibition of 95.2% at 30 min and temperature 50 ºC (Banožić et al., 2019). The purpose of this study was to determine the optimum UAE extraction conditions for the yield value of Wonosobo Robusta coffee bean extract with a temperature factor of 40, 50, 60 ºC and extraction time of 10, 30, 50 min using 96% ethanol solvent, The optimization yield data was then analyzed using RSM. The extract at the optimum conditions obtained will be continued by testing its anticancer activity against MCF-7 cancer cells using the Methyl Thiazole Tetrazolium (MTT) Assay method.

RESEARCH METHODS

Material and Equipment

The materials used include Wonosobo Robusta coffee beans (medium roasted), 96% ethanol, Michigan *Cancer Foundation*-7 (MCF-7 cancer cells), Phosphate Buffer Saline (PBS), *Dimethyl sulfoxide* (DMSO),*3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide reagent* (MTT reagent), *Dulbecco's Modified Eagle's Medium* (DMEM) *cell growth medium*, *Fetal Bovine Serum* (FBS) 5%; *Penicillin-Streptomycin* 1%, doxorubicin, dan trypsin. The tools used are sonicator, analytical balance, sieve mesh 40, furnace, crucible, oven, grinder, water bath, Biosafety Cabinet, CO_2 incubator, rotary evaporator, hemocytometer, inverted microscope, micropipette, centrifuge, 96-wells tissue culture plate, microplate reader, conical tube, flask, dropper pipette, and glassware.

Sample Preparation

Robusta coffee beans come from Wonosobo, Central Java, Indonesia. Plant determination is carried out at the Institute of Sciences Indonesian (LIPI), Bogor. Robusta green coffee beans as much as 1,992 g roasted with a medium roasting rate at 200 ºC for 15 min then dry sorting (Afriliana, 2018). Roasted coffee beans have been sorted, powdered with a grinder, and then sieved using a 40-mesh sieve. The coffee grounds are stored in a tightly closed container, given silica gel. Powder yield is calculated using equation (1).

$$\text{Percent extract yield} = \frac{\text{extract weights}}{\text{powder weight}} \times 100\% \qquad (1)$$

RSM Extraction Optimization

The extraction process was carried out based on the results of the Design Expert 7.1.5 Trial software analysis using a Central Composite Design (CCD)

experimental design with extraction temperature factors of 40, 50, 60 ºC and extraction time of 10, 30, 50 min. Obtained 13 combinations of extraction treatment.

A total of ±20 g of coffee powder was put into an Erlenmeyer and added with 96% ethanol solvent 200 mL (1:10), the Erlenmeyer was covered with aluminum foil. Samples were extracted using a sonicator according to the combination of treatments obtained with the wave frequency 40 kHz. The mixture is filtered, and the filtrate is concentrated on a water bath until thick extract was obtained. Furthermore, a verification test is carried out on the results of the RSM analysis**.**

Data processing

Extracted yield data (13 treatments) were processed by RSM analysis using Design software Expert 7.1.5 (Trial) with Central Composite Design (CCD) experimental design to determine the optimum conditions for extraction of RSM analysis on the influence of temperature and extraction time factors with the highest percent yield. The ANOVA test uses a 95% confidence level.

Extraction

A total of 300.0 g of coffee powder was put into an Erlenmeyer and added 96% ethanol solvent 3 L (1:10), the Erlenmeyer was closed using aluminum foil. The coffee grounds were extracted using a sonicator based on the optimization results of the RSM analysis that had been obtained, namely a temperature of 53^0C for 29.53 min with a wave frequency of 40 kHz. The mixture was filtered, and the filtrate was concentrated using a rotary evaporator. The concentrated filtrate was poured into a cup that had been tared and then evaporated over a water bath at a temperature of 70 ºC until a thick extract was obtained.

MTT Assay Cytotoxic Assay

Carefully weighed the extract as much as 8 mg, dissolved with adequate DMSO was added to the growth medium up to 1 mL, vortexed until homogeneous to obtain a solution with a concentration of 8000 µg/mL. The mother liquor was prepared by pipetting 0.25 mL of the sample solution with a concentration of 8000 g/mL and diluted with DMEM media to obtain a concentration of 500 µg/mL. From the mother liquor, 3 concentration series solutions were made, namely 25, 100, and 400 µg/mL which were used in the test. Cells without treatment were used as a negative control and doxorubicin as a positive control (Minarni et al., 2017).

MCF-7 cells that had grown confluently were sub-cultured by removing the cell media, adding 10 mL of PBS to clean the flask from the medium, and then discarding the PBS. Trypsin (0,125%) was added to the flask as much as 5 mL, incubated at 37 ºC for 5 min. The released cells were put into a 15 mL tube and then centrifuged at 1500 rpm for 5 min. The supernatant was discarded, and cells were prepared for testing purposes. Then it was incubated again in a CO_2 incubator with a concentration of 5%. Cells were grown on 96 wells tissue culture plate with the number of 5000 cells/well and incubated for 24 hours in growth medium at 37ºC and 5% CO_2. Robusta coffee bean samples at each concentration were added as much as 100 µL/well, untreated cells were included as control cells. Then incubated again for 48 hours. MTT compound was added and incubated for 4 hours at 37 ºC and 5% CO_2. The cell supernatant was discarded, the formazan crystals formed were dissolved in ethanol 70%. Optical density (OD) reading performed using a

microplate reader at a wavelength of 565 nm. The results of the absorbance readings were converted in percent inhibition of cells using equation (2) (Minarni et al., 2017).

$$\text{Percent inhibition} = \frac{\text{Absorbance of negatif control - Absorbance of sample}}{\text{Absorbance of negatif control}} \times 100\% \quad (2)$$

RESULT AND DISCUSSION

The coffee beans used in this study were robusta coffee beans on ripe coffee cherries which were marked by the outer skin of the coffee fruit being red when harvested, having a uniform size and free from insect bites or no defects. Plant determination was carried out at the Indonesian Institute of Sciences (LIPI), Bogor, in the form of roots, stems and leaves of the Robusta coffee plant. Determination aims to ensure the correctness of the type of plant used. The results of the determination of the three samples stated that the sample was a species of *Coffea canephora* belonging to the *Rubiaceae* tribe (Bicho et al., 2013).

The coffee powder obtained was 1695 g with a powder yield of 85,0903%. The characteristics of the coffee powder produced are brown, coarse powder, strong aromatic odour, and bitter taste.

Preliminary tests were carried out using temperature factors of 50, 60, 70 ºC and extraction time of 10, 30, 50 min. The selection of temperature and time levels was based on the research of (Goebel et al., 2016), Syakfanaya et al. (2019) and Banožić et al. (2019) modified. The results of the preliminary test showed that at a temperature of 50 ºC and a time of 30 min the highest extract yield was 18.1915%. The optimum time for the preliminary test is according to the research reference of Syakfanaya et al. (2019) in extracting caffeine and chlorogenic acid compounds from Robusta coffee beans. The preliminary test aims to determine the independent factor that has the most influence on the response as a basis for determining the right factor in RSM optimization.

Extraction optimization was carried out using a temperature factor of 40, 50, 60 ºC and a time factor of 10, 30, 50 min based on the results of the preliminary test. The results of the extraction optimization can be seen in Table 4. The data were analysed using Design Expert 7.1.5 (*Trial*) software using the RSM method with CCD design to determine the optimum extraction conditions. The RSM method was chosen because it can consider the influence of two or more factors in one experiment and can estimate the interaction between the factors used to make it easier to identify the factors that have the most significant influence on the response (Fajrin & Marchelina, 2017). CCD designs have better predictive quality with a smaller number of runs.

Table 1. Extraction Optimization Results

Temperature (ºC)	Time (Min)	Extract Yields (%)
50	50	8,6447
40	50	7,8888
50	30	9,1863
50	10	8,4879
60	30	8,5229
60	10	8,1144
40	30	7,3220
60	50	7,5595
40	10	6,4753
50	30	9,0280
50	30	9,2139
50	**30**	**9,3496**
50	30	9,0213

Based on Table 1, the highest extract yield was obtained at a temperature of 50 ºC for 30 min, namely 9.3496%. The optimum extraction conditions were obtained in accordance with the research of Banožić et al. (2019) in extracting

antioxidant compounds using the ultrasonic wave method. Extraction of *Gnetum gnemon* seeds using ultrasonic wave method showed no increase in extract yield for more than 30.18 min (Kunarto et al., 2019). At an extraction temperature of 40-50 ºC the yield of the extract increased because the movement of the ethanol molecules became faster and random which caused the cell pores to stretch so that the solvent easily entered the cells to extract metabolites and at a temperature of more than 50 ºC (Kristiningrum et al., 2016). The yield of the extract decreased because the temperature was too high causing damage to the compound and the solvent was reduced due to evaporation. At 10-30 min the extract yield increased with increasing extraction time because the contact time between the solvent and the sample was getting longer. At 50 min there was a decrease in the yield of the extract because the solvent had reached the saturation point and the extraction ability decreased. Ultrasonic wave-assisted extraction time that is too long can degrade phenolic components causing a decrease in yield value (Odabaş & Koca, 2016). The yield value is related to the effectiveness of the extraction and the amount of secondary metabolite content in a material (Abdeltaif et al., 2018), the higher the percentage yield, the more secondary metabolites contained, so the higher the activity and indicates the right extraction method (Dewatisari et al., 2018).

Based on Figure 1, the extract yield increased with increasing temperature and time to the optimum point which then decreased. Figure 2 is a cross-section of a 3D surface plot graph showing the extraction yield under various conditions visualized in different colours. The lowest to highest extract yields are shown in blue, green, yellow, orange to red. The results of the RSM optimization analysis are the temperature 52.74 ºC; time of 29.53 min resulted in an extract yield of 9.1621% which can be seen in Figure 2. The desirability value obtained is 1. Desirability indicates the degree of accuracy of the optimal solution suggested against a target that is worth 0 to 1 (Lee et al., 2018). The desirability value is getting better if it is close to 1 which indicates a high value of optimization accuracy.

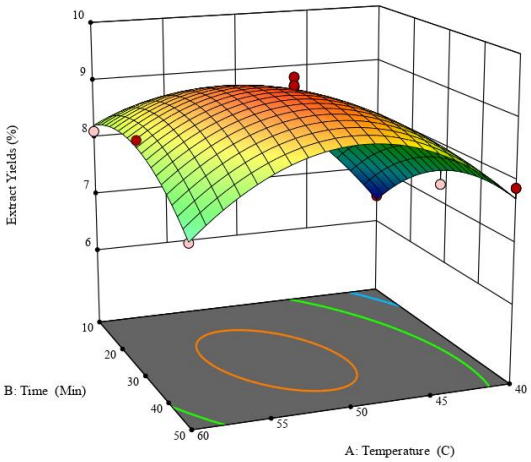

Figure 2. *Contour surface* 2D optimization extraction

The proof test is an act of extraction work directly in accordance with the alleged results of the RSM equation under optimal conditions. The results of the verification test can see the difference with the predicted response through the RSM equation. Based on the RSM equation, a value of 9.1621% was obtained, then verification was carried out and obtained a value of 9.1592 %. The experimental extract yield accuracy value to the predicted extract yield was 99.9683%, which indicated that the recommended model was suitable. The actual extract yield was still in the CI and PI ranges. The verification results, which are still within the CI and PI ranges, show that the solution suggested by RSM can be applied to real conditions with optimal results.

The results of the optimization of the extract yield with temperature and time factors were processed using Design Expert 7.0 Trial software RSM analysis

with CCD design. The ANOVA test in this study used a 95% confidence level with a value of = 0.05; so that the probability of the experimental truth level is 95% and the maximum error tolerance is 5%. Based on the results of the ANOVA analysis, the recommended model for measuring the response to factors is a quadratic model vs. the interaction of two factors. The selected model is the appropriate model and has a high level of significance as indicated by the significant model analysis results and the insignificant lack of fit value indicates the model is in accordance with the experimental data (Montgomery, 2013).

$$Y = 9.13 + 0{,}42A + 0.17B - 0.49\ A * B - 1.15\ A * A - 0.15\ B * B \quad (3)$$

Description: Y = Yield, A = Temperature, B = Time, A*B = Interaction of Temperature and Time, A*A (A2) = Time is square, B*B (B2) = Temperature square.

Equation 3 is the ANOVA test result equation which shows the increase in the percent yield of the extract is directly proportional to the increase in the temperature and time factors because it is positive, while the increase in the interaction factor of temperature with time and the temperature and time factors are increased twice will cause a decrease in the percent yield because it is negative. . The negative sign of the squared temperature factor and the time squared also shows a quadratic pattern

The R-Squared value obtained is 0.9751 which shows that time and temperature factors affect the extract yield of 97.51% and only 2.49% is influenced by other factors not included in the model. The R-Squared value which is getting closer to 1 indicates a close relationship between the independent factors and the response.

The suitability of the model can also be seen from the comparison of the actual value of the study with the prediction of the standard deviation of 0.18. A low standard deviation value indicates an accurate model. Figure 3 shows the predicted value which is described as a straight line and the actual value is depicted as a box that is not far in the straight-line area so that it can be said that the actual experimental data is normally distributed and does not have a significant difference with the predicted value.

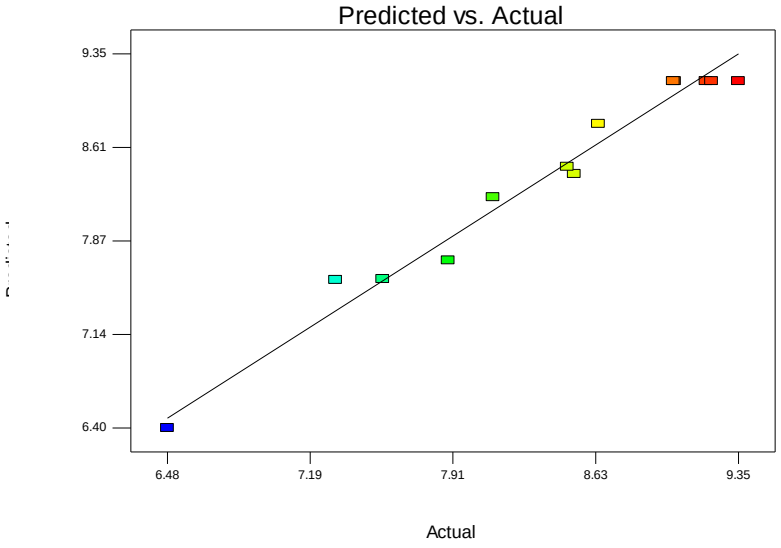

Figure 3. The relationship between the predicted value and the actual value of the extract yield

To determine whether there is an effect of time and temperature factors on the yield value, a comparison of the P-value with (0.05) is carried out. If the P-value < then the factor has a significant influence on the yield. Based on the results of the ANOVA analysis, it can be concluded that the factors that influence the yield of the extract include the temperature factor, the interaction of temperature and time, temperature^2 and time^2; while the time factor did not have a significant effect on the extract yield.

Anticancer activity tests were carried out on MCF-7 cells using the MTT method with 3 concentration variations, namely 25, 100, and 400 µg/mL (Minarni et al., 2017). The MTT method was chosen because it is a safe, accurate and most commonly used method for in vitro cytotoxicity and cell viability testing (Aslantürk, 2018).

In the test of Robusta coffee bean extract concentrations of 25 and 100 µg/mL did not show any inhibitory activity against MCF-7 cells, a concentration of 400 µg/mL resulted in 83.25% inhibition, the greater the concentration of the extract the greater the inhibitory activity produced. Based on the classification of the number of living cells, Robusta coffee bean extract with a concentration of 400 µg/mL was classified as strong cytotoxic because the living cells were less than 40% (López-García et al., 2014). The inhibitory activity of Wonosobo Robusta coffee bean extract against MCF-7 cells at a concentration of 400 g/mL was almost close to the percent inhibition of doxorubicin at a concentration of 400 g/mL, which was 88.71% (Table 2). According to research by Bravo et al.(2013) Robusta coffee bean extract at a concentration of 1000 µg/mL inhibited the growth of Hela cells by 60%; Caffeine compound at a concentration of 500 µg/mL has an antiproliferative activity on Huvec cells of 60.5% through an apoptotic mechanism (Li et al., 2013), the results of Bauer et al. (2018) reported that Robusta green coffee beans can inhibit more than 50% of prostate cancer cell growth at a concentration of 500 µg/mL. The presence of antiproliferative activity from Wonosobo Robusta coffee beans is thought to be due to the presence of active compounds such as chlorogenic acid, flavonoids, and caffeine which have anticancer activity (Rao & Nadumane, 2016; Gouthamchandra et al., 2017; Li et al., 2013) so it can be concluded that the extract of Wonosobo Robusta coffee beans has the potential as an anticancer but the resulting effect is influenced by the amount of concentration given (dose-dependent). In Figure 4, MCF-7 cells as control cells and cells treated with extracts at concentrations of 25 and 100 g/mL had the same morphology, namely round to oval in shape, colorless in the center of the cell and formed a clustered mass indicating living cells. MCF-7 cells that were treated with extracts at a concentration of 400 g/mL and most of the positive control cells experienced death which was indicated by a blackish colour in the nucleus and spread out.

Table 2. The inhibitory activity of Wonosobo Robusta coffee bean extract against MCF-7 Cells

Groups	%Inhibition			
	Negative Control	**Concentration 25µg/mL**	**Concentration 100µg/mL**	**Concentration 400µg/mL**
Extracts	0,00^b	-16,15^a	-14,14^a	83,25^c
Doxorubicin	0,00^b	95,25^c	92,75^c	88,71^c

* Letter differences indicate a significant difference in p<5%

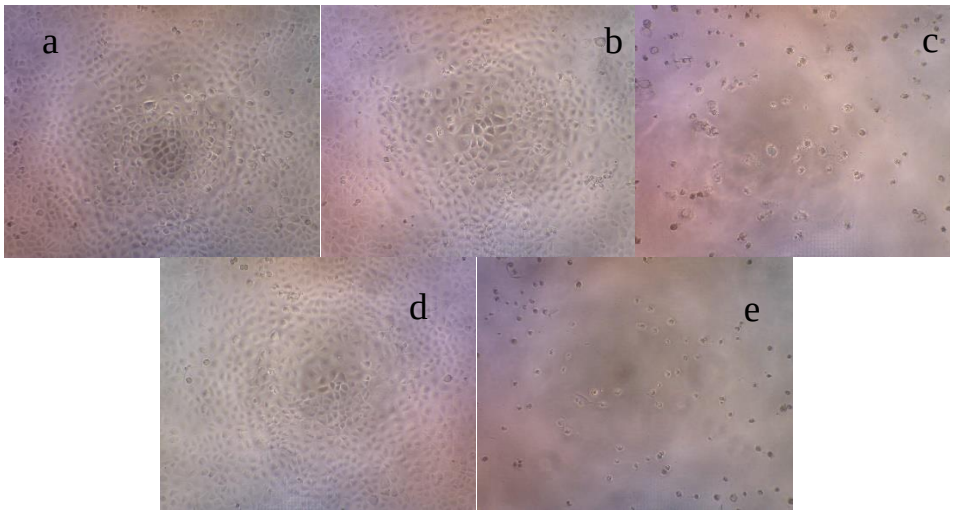

Figure 4. MCF-7 cell morphology. Extract treatment 25 µg/mL (a), extract treatment 100 µg/mL (b), extract treatment 400 µg/mL (c), control cells (d), doxorubicin control 25 µg/mL (e).

These results indicate that there are ingredients in coffee that act as anti-oxidants (Górecki & Hallmann, 2020) like polyphenols (Król et al., 2020), functional ingredient content of coffee described by (Haile & Kang, 2019), while the benefits of coffee in overcoming cancer are explained by (Gouthamchandra et al., 2017). The role of coffee as a sedative has been investigated by Mikami & Yamazawa (2015). The more detailed study of relieving mechanical and cold hyperalgesia in a rat model of neuropathic pain has been provided by Hara et al. (2014).

Doxorubicin is used widely as a breast cancer therapy but long-term use can cause resistance caused by the high amount of PgP and Bcl-2 in MCF-7 cells. PgP is found in cell membranes which is a multidrug resistance (MDR) protein that can reject drug compounds from entering cells, while Bcl-2 is a class of proteins that have antiapoptotic activity that inhibits apoptosis or programmed cell death (Risdian et al., 2014). Doxorubicin can also cause cardiotoxicity due to the accumulation of iron in the mitochondria during walking therapy (Ichikawa et al., 2014; Damiani et al., 2016). Therefore, the search for natural anticancer drug compounds with minimal side effects is important to be carried out and researched.

CONCLUSION

A temperature of 52.74 ºC and a time of 29.53 min is the optimum condition for ultrasonic wave-assisted extraction to the percent yield of Wonosobo Robusta coffee bean extract because of RSM optimization analysis. The yield of 9.16% was obtained. Wonosobo Robusta coffee bean extract at a concentration of 400 µg/mL has antiproliferative activity against MCF-7 cancer cells of 83.25% so that it is anticancer.

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REFERENCES

Abdeltaif, S. A., Sirelkhatim, K. A., & Hassan, A. B. (2018). Estimation of phenolic and flavonoid compounds and antioxidant activity of spent

coffee and black tea (Processing) waste for potential recovery and reuse in Sudan. *Recycling*, *3*(2). https://doi.org/10.3390/recycling3020027

Afriliana, A. (2018). *Teknologi Pengolahan Kopi Terkini*. Deepublish. https://books.google.co.id/books?id=RFlVDwAAQBAJ

Aslantürk, Ö. S. (2018). In Vitro Cytotoxicity and Cell Viability Assays: Principles, Advantages, and Disadvantages. *Genotoxicity - A Predictable Risk to Our Actual World*, 1–18. https://doi.org/10.5772/intechopen.71923

Banožić, M., Banjari, I., Jakovljević, M., Šubarić, D., Tomas, S., Babić, J., & Jokić, S. (2019). Optimization of ultrasound-assisted extraction of some bioactive compounds from tobacco waste. *Molecules*, *24*(8). https://doi.org/10.3390/molecules24081611

Bauer, D., Abreu, J., Jordão, N., da Rosa, J. S., Freitas-Silva, O., & Teodoro, A. (2018). Effect of roasting levels and drying process of coffea canephora on the quality of bioactive compounds and cytotoxicity. *International Journal of Molecular Sciences*, *19*(11). https://doi.org/10.3390/ijms19113407

Bicho, N. C., Leitão, A. E., Ramalho, J. C., De Alvarenga, N. B., & Lidon, F. C. (2013). Identification of chemical clusters discriminators of Arabica and Robusta green coffee. *International Journal of Food Properties*, *16*(4), 895–904. https://doi.org/10.1080/10942912.2011.573114

Bravo, J., Arbillaga, L., de Peña, M. P., & Cid, C. (2013). Antioxidant and genoprotective effects of spent coffee extracts in human cells. *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association*, *60*, 397–403. https://doi.org/10.1016/j.fct.2013.08.002

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *68*(6), 394–424. https://doi.org/https://doi.org/10.3322/caac.21492

Damiani, R. M., Moura, D. J., Viau, C. M., Caceres, R. A., Henriques, J. A. P., & Saffi, J. (2016). Pathways of cardiac toxicity: comparison between chemotherapeutic drugs doxorubicin and mitoxantrone. *Archives of Toxicology*, *90*(9), 2063–2076. https://doi.org/10.1007/s00204-016-1759-y

Dewatisari, W. F., Rumiyanti, L., & Rakhmawati, I. (2018). Rendemen dan Skrining Fitokimia pada Ekstrak Daun Sanseviera sp. *Jurnal Penelitian Pertanian Terapan*, *17*(3), 197. https://doi.org/10.25181/jppt.v17i3.336

Fajrin, J., & Marchelina, N. (2017). *Aplikasi Metode Eksperimen Response*. *13*(2), 79–90.

Goebel H., Gustavo Ariel Rostagno, Maurico Martínez, J., da Fonseca Machado, A. P., & Garcia Mendoza, M. del P. (2016). Ultrasound-assisted extraction of antioxidants present in roasted coffee beans (Coffea Arábica L.). *XXIV Congresso de Iniciação Científica Da Unicamp*, *I*, 50813. https://doi.org/10.19146/pibic-

2016-50813

Górecki, M., & Hallmann, E. (2020). The antioxidant content of coffee and its in vitro activity as an effect of its production method and roasting and brewing time. *Antioxidants*, *9*(4). https://doi.org/10.3390/antiox9040308

Gouthamchandra, K., Sudeep, H. V, Venkatesh, B. J., & Shyam Prasad, K. (2017). Chlorogenic acid complex (CGA7), standardized extract from green coffee beans exerts anticancer effects against cultured human colon cancer HCT-116 cells. *Food Science and Human Wellness*, *6*(3), 147–153. https://doi.org/https://doi.org/10.1016/j.fshw.2017.06.001

Haile, M., & Kang, W. H. (2019). Antioxidant activity, total polyphenol, flavonoid and tannin contents of fermented green coffee beans with selected yeasts. *Fermentation*, *5*(1). https://doi.org/10.3390/fermentation5010029

Hara, K., Haranishi, Y., Kataoka, K., Takahashi, Y., Terada, T., Nakamura, M., & Sata, T. (2014). Chlorogenic acid administered intrathecally alleviates mechanical and cold hyperalgesia in a rat neuropathic pain model. *European Journal of Pharmacology*, *723*, 459–464. https://doi.org/https://doi.org/10.1016/j.ejphar.2013.10.046

Kristiningrum, N., Cahyanti, Y. N., Wulandari, L., Farmasi, F., & Jember, U. (2016). Determination of Total Phenolic Content and Antioxidant Activity in Methanolic Extract of Robusta and Arabica Coffee Leaves. *Proceeding ICMHS*, 96–99.

Król, K., Gantner, M., Tatarak, A., & Hallmann, E. (2020). The content of polyphenols in coffee beans as roasting, origin and storage effect. *European Food Research and Technology*, *246*(1), 33–39. https://doi.org/10.1007/s00217-019-03388-9

Kunarto, B., Sutardi, S., Supriyanto, S., & Anwar, C. (2019). Optimasi Ekstraksi Berbantu Gelombang Ultrasonik pada Biji Melinjo Kerikil (Gnetum gnemon L., 'Kerikil') Menggunakan Response Surface Methodology. *Jurnal Aplikasi Teknologi Pangan*, *8*(3), 104. https://doi.org/10.17728/jatp.5122

Lee, D. H., Jeong, I. J., & Kim, K. J. (2018). A desirability function method for optimizing mean and variability of multiple responses using a posterior preference articulation approach. *Quality and Reliability Engineering International*, *34*(3), 360–376. https://doi.org/10.1002/qre.2258

Li, H., Jin, S.-Y., Son, H.-J., Seo, J. H., & Jeong, G.-B. (2013). Caffeine-induced endothelial cell death and the inhibition of angiogenesis. *Anatomy & Cell Biology*, *46*(1), 57. https://doi.org/10.5115/acb.2013.46.1.57

López-García, J., Lehocký, M., Humpolíček, P., & Sáha, P. (2014). HaCaT Keratinocytes Response on Antimicrobial Atelocollagen Substrates: Extent of Cytotoxicity, Cell Viability and Proliferation. *Journal of Functional Biomaterials*, *5*(2), 43–57. https://doi.org/10.3390/jfb5020043

Mikami, Y., & Yamazawa, T. (2015). Chlorogenic acid, a polyphenol in coffee, protects neurons against glutamate neurotoxicity. *Life Sciences*, *139*, 69–74. https://doi.org/https://doi.org/10.1016/j.lfs.2015.08.005

Minarni, Artika, I. M., Julistiono, H.,

Bermawie, N., Riyanti, E. I., Hasim, & Hasan, A. E. Z. (2017). Anticancer activity test of ethyl acetate extract of endophytic fungi isolated from soursop leaf (Annona muricata L.). *Asian Pacific Journal of Tropical Medicine*, *10*(6), 566–571. https://doi.org/10.1016/j.apjtm.2017.06.004

Ministry of Health of the Republic of Indonesia. (2015). *INFODATIN: Situasi Penyakit Kanker*. https://pusdatin.kemkes.go.id/article/view/15090700004/situasi-penyakit-kanker.html

Montgomery, D. C. (2013). Design and Analysis of Experiments Eighth Edition. Arizona State University. In *Copyright* (Vol. 2009, Issue 2005).

Odabaş, H. İ., & Koca, I. (2016). Application of response surface methodology for optimizing the recovery of phenolic compounds from hazelnut skin using different extraction methods. *Industrial Crops and Products*, *91*, 114–124. https://doi.org/https://doi.org/10.1016/j.indcrop.2016.05.033

Rao, S., & Nadumane, V. K. (2016). Evaluation of the anticancer potential of coffee beans: An in vitro study. *Indian Journal of Traditional Knowledge*, *15*(2), 266–271.

Risdian, C., Mozef, T., & Lotulung, P. D. . (2014). -. In *Isolasi Siklokomunol dari Daun Sukun Artocarpus altilis (PARKINSON) FOSBERG serta Aktivitasnya sebagai Antikanker* (Vol. 16, pp. 82–86).

Safdar, M. N., Kausar, T., & Nadeem, M. (2017). Comparison of Ultrasound and Maceration Techniques for the Extraction of Polyphenols from the Mango Peel. *Journal of Food Processing and Preservation*, *41*(4), e13028. https://doi.org/https://doi.org/10.1111/jfpp.13028

Shashni, B., Sharma, K., Singh, R., Sakharkar, K. R., Dhillon, S. K., Nagasaki, Y., & Sakharkar, M. K. (2022). Retraction Note to: Coffee component hydroxyl hydroquinone (HHQ) as a putative ligand for PPAR gamma and implications in breast cancer (BMC Genomics, (2013), 14, (S6), 10.1186/1471-2164-14-S5-S6). *BMC Genomics*, *23*(1). https://doi.org/10.1186/s12864-022-08371-5

Sholihah, M. (2017). Aplikasi Gelombang Ultrasonik untuk Meningkatkan Rendemen Ekstraksi dan Efektivitas Antioksi dan Kulit Manggis. *Jurnal Keteknikan Pertanian*, *5*(2), 161–168.

Syakfanaya, A. M., Saputri, F. C., & Mun'im, A. (2019). Simultaneously extraction of caffeine and chlorogenic acid from Coffea canephora bean using natural deep eutectic solvent-based ultrasonic assisted extraction. *Pharmacognosy Journal*, *11*(2), 267–271. https://doi.org/10.5530/pj.2019.11.41

Utami, N. F., Nhestricia, N., & Maryanti, S. (2018). Antioxidant Activity Test from Robusta Coffee Seeds (Coffea canephora P.) Based On High Flat Ecology Differences in Java Island. *Fitofarmaka*, *8*(1), 60–65.