Research Article
DOI : 10.33751/jf.v13i2.8922

FITOFARMAKA: Jurnal Ilmiah Farmasi
Vol.13, No.2, December 2023 : 105-114
p-ISSN : 2087-9164 e-ISSN : 2622-755X

Potential of Malang Robusta Coffee Beans as Anti-breast Cancer *In-Vitro*

Novi Fajar Utami[1,2*], Berna Elya[1], Hayun Hayun[3], Kusmardi Kusmardi[4,5,6], Syamsu Nur[7], Annisa Fajriati[2]

[1]Department of Phytochemistry and Pharmacognosy, Faculty of Pharmacy, Universitas Indonesia, Depok 16424 West Java, Indonesia
[2]Faculty of Math and Science, Universitas Pakuan, Jl. Raya Pakuan 1 Bogor, Indonesia
[3]Faculty of Pharmacy, Universitas Indonesia, Depok 16424 West Java, Indonesia
[4]Department of Anatomic Pathology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta, 10430, Jakarta, Indonesia, 10430 Indonesia.
[5]Drug Development Research Cluster, Indonesia Medical Educational and Research Institute, Jl. Salemba Raya No.6, Jakarta 10340, Indonesia
[6]Human Cancer Research Cluster, Indonesia Medical Educational and Research Institute, Jl. Salemba Raya No.6, Jakarta 10340, Indonesia
[7]Department of Pharmaceutical Chemistry, Almarisah Madani University, Makassar, South Sulawesi, Indonesia

**Corresponding Author Email: novi.unpak@gmail.com*

Submit: September 20th, 2023 Revised: December 4th, 2023 Accept: December 16th, 2023

ABSTRACT

Robusta coffee from Malang Regency, East Java is one of the superior products of the largest coffee-producing region in Indonesia. The coffee has high antioxidants. The component that acts as an antioxidant is the tannin content, and these tannins are also anti-cancer. Using the UV-Vis spectrophotometry method the tannin content in Malang Robusta coffee beans is known and then to measure its ability as an anticancer in-vitro against breast cancer cells using MCF-7. The results showed that the tannin contained in Malang Robusta coffee bean extract was 2.81 % and inhibited MCF-7 at 400 µg/mL, it has potential as an anticancer with an inhibition value of 68.88 %. Therefore, the use of Robusta coffee from Malang at this concentration can be used as an anti-breast cancer agent.

Keywords: *Robusta*; *anticancer*; *MCF-7; in-vitro*

INTRODUCTION

According to the Central Bureau of Statistics (2018) coffee production in Indonesia has been increasing steadily until now it has a coffee plantation area of 1,235,798 hectares with a total coffee production of around 713,921 tons per year and places East Java as the province with largest production output. One of the most coffee-producing regions in East Java is Malang with its superior product, namely robusta coffee originating from Malang district with a total annual production of 2,387 tons covering an area of 3,373 hectares (BPS-Statistics of Malang Regency, 2018). In addition, Malang robusta coffee has the highest antioxidant activity among other regions in East Java with an IC50 value of 37.47 % (Utami et al., 2018).

Robusta coffee beans are known to contain compounds such as alkaloids, flavonoids, saponins, tannins, caffeine, and phenols (Muttaqin et al., 2022). Humin acid, protein, minerals, chlorogenic acid, fat,

trigonelline, aliphatic acids (Kiyama, 2019), melatonin and serotonin (Ramakrishna et al., 2012), and isoflavones (Alves et al., 2010). Of the many compounds contained in coffee beans, one of the most efficacious antioxidants is tannins.

According to Hutami et al. (2018) research, the tannin content in Aceh Gayo arabica coffee is 2.56 %. A study by (Istiqomah et al., 2015) stated that the tannin isolate of the air fraction from bamboo grass plants had anticancer activity in IC50 value of 2.046 µg/mL to T47D cells. Arabica coffee at a concentration of 0.1 µg/mL has a percent viability of 33.3 % in HeLa cells (Rao & Nadumane, 2016). Robusta coffee at a concentration of 100 µg/mL has a viability percentage of 48.74 % in HT29 cells (Polamuri et al., 2020). Chlorogenic acid is a component that has the potential as an anticancer (Gouthamchandra et al., 2017), but flavonoid compounds also play a role (Rao & Nadumane, 2016), and hydroxy hydroquinone compounds according to Shashni et al. (2022) acts as an anticancer compound, hydroxy hydroquinone is a natural constituent of coffee accounting for main dry matter constituent in roasted beans. The study states that hydroxy hydroquinone was observed to dock and form hydrogen bonds with PDB ID - 2PRG (3-D PDB crystal structure of the ligand binding domain of the human peroxisome proliferator-activated gamma receptor solved in complexation with Rosiglitazone, a PPAR gamma agonist/ligand). Coffee component hydroxy hydroquinone as a potential ligand for PPARg and its role in induction of apoptosis in breast cancer cells by delineating the glycolytic pathway gene regulation by PPARg activation.

The second leading cause of death for women in Indonesia is breast cancer. According to Bray et al. (2018), there are 22,693 deaths due to cancer and a total of 58,256 new cases each year. Judging from the tannin content which has the potential as an anti-breast cancer, it is necessary to test the Robusta coffee extract originating from Malang Regency, East Java.

METHODS

Material and Equipment

Malang Robusta coffee beans with medium roast which have been determinated in The Institute of Sciences Indonesian (LIPI), Bogor on January 21, 2020 MCF-7 cancer cells, DMSO, Phosphate Buffer Saline (PBS), 96 % ethanol, 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT reagent), medium Dulbecco's Modified Eagle's Medium (DMEM) for cell growth, distilled water, Fetal Bovine Serum (FBS) 5 %; trypsin, Penicillin-Streptomycin 1%, and doxorubicin. Tools are used biosafety cabinet level-2 (Nuaire®), analytical balance (LabPro-DT224C®), furnace, sieve 40 mesh, grinder, oven, water bath, *inverted microscope* (Nikon®), incubator CO_2 (Binder®), rotary evaporator, hemocytometer, centrifuge, 96 *well tissue culture plate* (Corning®), flask T25 (Corning®), microplate reader, and conical tube.

Process of Producing Coffee Powder

Robusta coffee beans obtained are sorted, cleaned of impurities and weighed. After that, roasting is carried out at medium pressure with a temperature of 200 ^oC for 10 minutes and then ground to a coarse powder. Then sieved with a 40-mesh sieve and then weighed to get the final weight. The coffee powder is then stored in a dry, clean, and airtight container.

Extract Making

The coffee powder (300 g) was put in a dark bottle, then added with 3 L of 96 % ethanol solvent. The bottle is stored for 3 x 24 hours in a place protected from sunlight. For the first 6 hours, the coffee powder was soaked in ethanol solvent with occasional shaking, then left to stand for 18 hours. The coffee solution then filtered to separate the grounds and the filtrate. The filtrate obtained was then dried using a rotary evaporator until a thick extract was obtained (Ministry of Health, 2013). The yield of the extract is calculated using the formula 1.

$$\text{Percent extract yield} = \frac{\text{extract weights}}{\text{powder weight}} \times 100\ \% \qquad (2)$$

Moisture and Ash Content

The moisture content and ash content in robusta coffee beans ware determined according to the modified methods of Ministry of Health.

Phytochemical Test

Identification of group compounds contained in the coffee extract such as alkaloids, flavonoids, tannins and saponins was carried out based on modified method of Bungi & Dunggio, (2022).

Determination of Tannin Levels (Gurning et al., 2021)

Robusta coffee extract (10 mg) was dissolved with 10 mL of aquadest (1000 ppm). A total of 5 mL of solution was taken and then dissolved with aquadest in volumetric flask until the reach the mark to obtain 500 ppm. Take 1 mL and then add 0.4 mL of Follin's phenol reagent and allow to stand for 3 minutes then add 4 mL of saturated Na_2CO_3 solution and add water distillation to the limit mark and shake. Incubated for 20 minutes. Then read the absorption at a maximum wavelength of 745.5 nm.

MTT Assay Cytotoxic Assay (Minarni et al., 2017)

Tests to determine the cytotoxicity of coffee extracts began with the preparation of subcultures of MCF-7 cells. The result of incubated cells at 37 ºC for 5 minutes from the addition of trypsin (0.125 %) and with a 5 mL flask that had been separated from the substrate was centrifuged for 5 minutes at 1500 rpm. Cells were then prepared according to the needs of the test after being separated from the supernatant.,

A total of 5000 cells/well that have been grown and incubated in growth media for 24 hours at 37 ºC and 5 % CO_2 are then added to each concentration of 100 µL/well. Unused cells in the treatment will be incubated for 48 hours and used as cell control. MTT compound was added then incubated for 24 hours at 37 ºC and 5 % CO_2. The cell supernatant was discarded, the formazan crystals formed were then dissolved using 70 % ethanol as much as 100 µL/well. Optical Density (OD) was read using a microplate reader at a wavelength of 565 nm. (Minarni et al,2017). Using equation 2, the percentage of coffee inhibition against MCF-7 cells was determined.

$$\text{Inhibition} = \frac{\text{Abs. Negatif Control-Abs. Sample}}{\text{powderAbs(Negatif Control)}} \times 100\%$$

RESULT AND DISCUSSION

Research conducted by Bicho et al. (2013) and Hasan et al. (2021) shows that the coffee used in this study comes from the species *Coffea canephora*, which belongs to the *Rubiaceae* tribeand includes robusta type coffee beans originating from Malang, East Java. Factors such as the type of coffee used and the coffee producing region can cause variations in the results obtained, the process of preparing coffee bean samples into coffee powder also affects some test results and compound content. The roasting process with a medium roast maturity level produces compounds that give coffee its distinctive aroma and flavour (Fadri et al., 2020). The roasting process also reduces moisture content and converts sugars into CO_2, which acts as a transport medium for coffee aroma (Amit et al., 2017). Grinding and sieving at 40 mesh results in coffee powder with a smaller and more uniform particle size (Shanmugam, 2015). This increases the surface area of the powder in contact with the solvent, allowing more active substances to be extracted. The smaller particle size also improves the flavour and aroma of the coffee when brewed, as more coffee ingredients are dissolved (Cordoba et al., 2020).. The yield of coffee powder obtained in this research was 91.3410 %, which differed from the results obtained by Zhang et al. (2021) (around 84.6 %) and Juarez et al. (2018) (around 96.9 %). Factors such as the type of coffee used, coffee-producing areas, and yield losses in the milling process can cause variations in the results obtained, according to Felhi (2017), powder yield is influenced by various processes such as high temperature drying which causes water migration from the material to the environment, sieving which causes some particles to be trapped in the filter media. The organoleptic test revealed that the Malang

Robusta coffee bean powder was blackish-brown in color, with a bitter taste and a distinctive coffee aroma, which is consistent with the findings of Utami (2020). The coffee grounds can be seen in Figure 1.

Figure 1. Malang Robusta Coffee Powder.

To obtain extracts from coffee powder, an extraction process is carried out to transfer the components contained in the coffee powder into the solvent.. The transfer is done by attracting the components that dissolve in the solvent, which results in a transfer into the solvent through the interfacial layer and diffusion (Amit et al., 2017). The maceration extraction method is used because it is easy to do, uses simple tools, and does not require heating when withdrawing active substances (Zhang et al., 2018). According to Khobibah et al. (2022) this method is very suitable for samples that are not resistant to heating. Ethanol 96 % is used as a solvent because it can dissolve polar, semi-polar, and non-polar compounds.. This solvent is expected to dissolve the tannins in Robusta coffee completely (Hamboroputro and Yuniawati, 2017). Additionally, 96 % ethanol is used to prevent microbial growth in the extract (Khobibah et al., 2022). Soaking and shaking the powder in the solvent every 6 hours to ensure an even concentration inside and outside the cell, thus allowing optimal dissolution of the chemical content and complete extraction of substances. The extract obtained is a thick extract resulting from the thickening process with a rotary evaporator in the form of a thick blackish brown material with a distinctive coffee aroma.. Figure 2 shows the Malang robusta coffee condensed extract.

The extract yield from this study was 10.3392 %, this result is higher than the results of research conducted by Utami (2020) which stated that the yield of extracts of poor robusta coffee beans was 8.39 %, and higher than the results of Hasan's research (2022) where the extraction of wonosobo coffee beans produced the highest yield of 9.34 %. This result is also higher than the research conducted on Malang robusta coffee which obtained a yield of 3.64 % for the extract (Rejo et al., 2020).This difference in results is influenced by several factors such as differences in extraction methods, simplicial size, amount of simplicial, and solvents used (Fatimah et al., 2019).

Figure 2. Malang Robusta Coffee Extract.

This difference in coffee levels can be caused by the size and quality of the coffee beans used. Coffee beans that are defective in size and of low quality cause the coffee beans to absorb more water. This is because the cell network in coffee is not perfect, which makes the empty volume in it more, causing the coffee to expand and produce high water content (Rejo, et al., 2020).

The ash content of Malang coffee powder and extract met the standards of the Ministry of Health (2013), was less than 10.2 %, with the ash content of the powder being 3.6812 ± 0.0639 and the extract being 5.6849 ± 0.0766.. These results are also lower than Utami, (2020), namely for the powder ash content of 5.93 % while the extract is 8.26%.This difference is caused by the location of planting, quality and mineral content. The high mineral content and the cleanliness of the coffee beans from the remaining epidermis as well as dirt will increase the ash content (Rejo, et al., 2020).

Compounds tested on powder and condensed extract of Malang robusta coffee beans included tannins, alkaloids, saponin, and flavonoids (Table 1). The results of the phytochemical test of powder and extracts are in

accordance with research conducted by Utami et al. (2018). which stated that robusta coffee from the Malang area contains alkaloids, flavonoids, tannins and saponins. In the qualitative test of tannins using $FeCl_3$ reagent, the sample showed positive results with the results of the solution also having a greenish-black precipitate and a red precipitate which refers to the identification of condensed tannins. This qualitative test indicates that there is a class of compounds contained in a sample (Utami et al., 2018).

The determination of the wavelength of tannic acid was obtained at 745.5 nm. These results were not much different from the research by Pratama et al. (2019) who carried out the determination of tannin content in black cumin seeds using tannic acid and Follin's chiocalteu reagent with a yield of 740 nm.. From the data above, it can be seen that the tannin content obtained by robusta coffee in the Malang area was 2.81 %, this result was greater than that of Hutami et al. (2018) study using Aceh Gayo Arabica coffee which obtained a tannin content of 2.56 %. This difference is due to differences in the type of coffee, where it grows, climate, nutrients, temperature, and light intensity (Ahmed et al., 2021).

Compounds	Reagents	Powder	Extract
Alkaloids	Bourchardat	+	+
	Mayer	+	+
	Dragendorff	+	+
Flavonoids	Mg	+	+
	Zn	+	+
Tannins	$FeCl_3$	+	+
Saponins	HCl	+	+

The next test, which is in the anticancer test, MCF-7 cells are the most frequently used breast cancer cell model in vitro because they can express p53 wild type so they are sensitive to antineoplastic agents.(Cheng et al., 2022). The method used for anticancer testing is the MTT assay, the principle of this method, which is staining to see the reaction between living cells with MTT to form purple formazan fibres and if it does not react it will not cause colour and after that it is read with an ELISA reader (microplate reader). (Galán-Huerta et al., 2016). The results of the MCF-7 cells were compared with the cells with the sample treatment, namely Malang robusta coffee bean extract and the positive control, namely Doxorubicin. Figure 3 is the result of the MCF-7 cell anticancer activity test.

Table 1. Phytochemical Test Results

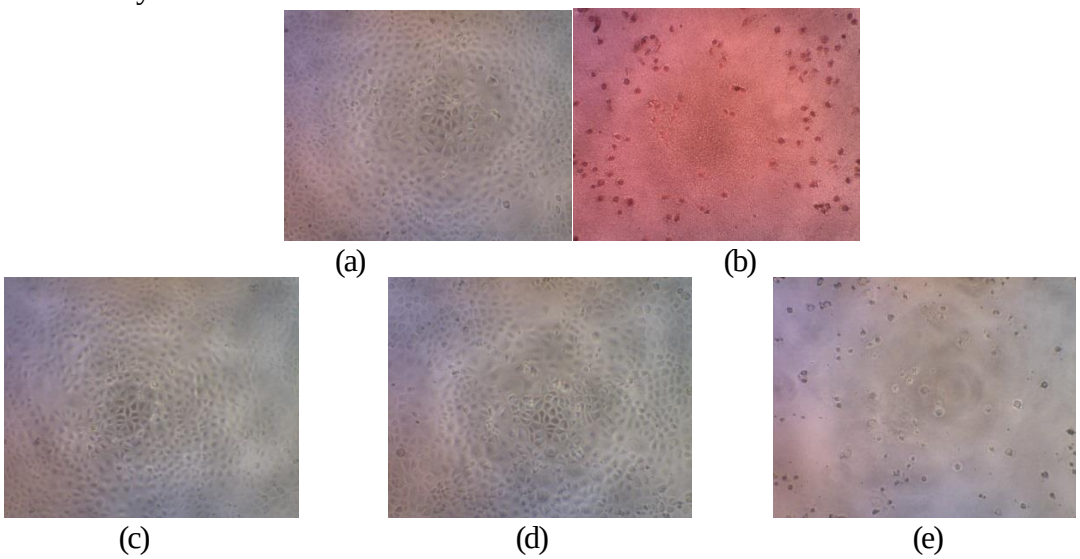

Figure 3. (a) Control cells), (b) Doxorubicin 400 µg/mL (positive control), (c), (d), (e) treatment with concentration 25 µg/mL, 100 µg/mL and 400 µg/mL sequentially

From Figure 3, it can be seen that the MCF-7 cells treated with the extract underwent apoptosis with characteristics such as shrinkage and cytoplasmic compaction accompanied by damage to the extracellular matrix. So that its activity as a cytotoxic works through the induction of apoptosis and inhibition of cell migration which leads to anti-metastatic (Chaudhry et al., 2022). According to minarni (2017) living cells were spheroidal and pellucid in the middle, while dead cells have an irregular shape and blue colour, and devoid of nucleus. Cancer cells that are still alive will be marked with an epithelial shape and have a light colour, while dead cells will be marked with a round shape with a dark or non-luminous colour (Sirait et al., 2019).

Doxorubicin as a positive control on MCF-7 cells inhibited by 88.72 % at a concentration of 400 µg/mL while the treatment with Malang robusta coffee bean extract samples was made into three concentrations namely 25, 100, and 400 µg/mL respectively produce % inhibition of -6.06; -11.28; and 68.88 %. The resulting data is a negative number because it allows the concentration used to be small, and this indicates that the sample at that concentration cannot inhibit oxidation activity because the % inhibition produced does not meet the requirements and does not produce anticancer activity. So, it can be interpreted that of the three positive control concentrations that used doxorubicin, the inhibition percentage was classified as moderate to strong at 88.72 % but with a concentration of 400 µg/mL, so that its bioactivity against MCF-7 breast cancer cells could be declared active. This result is not much different from the research conducted by Sirait et al. (2019) that doxorubicin produces an inhibition percentage of 92.26 % at a concentration of 3 ppm which is classified as moderate to strong so that it is active against MCF-7 breast cells. Then, test samples from cells that were treated with coffee extract up to 100 µg/mL had weak bioactivity so that it could be said to be inactive against MCF-7 breast cancer cells while at concentrations of 400 µg/mL had moderate to strong bioactivity. so that at this concentration the sample is said to be active against MCF-7 but less effective because the concentration is quite large.

The results of cell treatment with Malang Robusta coffee bean extract up to 100 µg/mL can be said to have no anticancer potential in MCF-7 cells. The process of angiogenesis of cancer cells will excrete growth substances thereby stimulating the formation of new blood tissue for the growth and development of cancer cells. The greater the concentration value of the extract, the less the number of growing blood vessels so that cancer cells will die because they do not get enough nutrition. So that at a concentration of 400 µg/mL Malang Robusta coffee bean extract only has benefits as anti-angiogenesis in MCF-7 cancer cells. This is in line with research conducted by Polamuri et al. (2020) who stated that Robusta coffee has potential as an anticancer colon in HT29 cells with a percent viability of 67.91 % at a concentration up to 100 µg/mL it has a viability of 48.74 %. According to research by Rao & Nadumane (2016), Arabica coffee at a concentration of 0.1 µg/mL has a viability percentage of 33.3 % in HeLa cells (cervical cancer). López-García et al. (2014) states that living cells which are more than 80 % are in the non-cytotoxic level, the range of 80-60 % is classified as weak cytotoxic, the range of 60-40 % is classified as moderate cytotoxic, and live cells which are less than 40 % are included in the high cytotoxic level.

Coffee contains compounds such as polyphenols, then isomeric forms of catechins and galocatechins are flana-3-ol polymeric forms of tannins condensed from roasted coffee beans. (Krol et al., 2020; Latos-Brozio & Masek, 2020). The active compounds such as flavonoids, chlorogenic acid, and caffeine which have anticancer activity (Rao & Nadumane, 2016; Gouthamchandra et al. (2017). The Bravo et al. (2013) state the extract of coffee are inhibited Hela cell growth 60% at a concentration of 1000 g/mL. The antiproliferative activity of Huvec cells from caffeine compounds at a concentration of 500 g/mL was 60.5 % (Li et al., 2013). So it can be concluded that coffee has the

potential to be cytotoxic against MCF-7 of cancer cells at a 400 µg/mL with 68,88 % inhibition, some of which are classified as moderate to strong based on testing using the MTT Assay.

CONCLUSION

The tannin content of coffee bean robusta from Malang is 2.8 % with an inhibition value of 68.88 % at a concentration of 400 µg/mL *in vitro*. It can be concluded that the robusta coffee bean extract from Malang has a potential as anti-breast cancer which act as antiangiogenesis in MCF-7 cell line.

REFERENCES

Ahmed, S., Brinkley, S., Smith, E., Sela, A., Theisen, M., Thibodeau, C., Warne, T., Anderson, E., Van Dusen, N., Giuliano, P., Ionescu, K. E., & Cash, S. B. (2021). Climate Change and Coffee Quality: Systematic Review on the Effects of Environmental and Management Variation on Secondary Metabolites and Sensory Attributes of Coffea arabica and Coffea canephora. *Frontiers in Plant Science*, *12*(October), 1–20. https://doi.org/10.3389/fpls.2021.708013

Alves, R. C., Almeida, I. M. C., Casal, S., & Oliveira, M. B. P. P. (2010). Isoflavones in coffee: influence of species, roast degree, and brewing method. *Journal of Agricultural and Food Chemistry*, *58*(5), 3002–3007. https://doi.org/10.1021/jf9039205

Amin Rejo, Sri Rahayu, T. P. (2020). Karakteristik Mutu Biji Kopi Pada Proses Dekafeinasi. *Jurusan Teknologi Pertanian*, *1*(1), 69–73.

Amit, S. K., Uddin, M. M., Rahman, R., Islam, S. M. R., & Khan, M. S. (2017). A review on mechanisms and commercial aspects of food preservation and processing. *Agriculture and Food Security*, *6*(1), 1–22. https://doi.org/10.1186/s40066-017-0130-8

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

BPS-Statistics of Malang Regency. (2018). *Malang Regency in Fidures*. BPS-Statistics of Malang Regency. https://doi.org/1102001.3507

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

Bungi, S. Y. K., & Dunggio, S. D. (2022). Identification Of Chemical Compounds From The Simplisia Of Jackfruit Leaves (Artocarpus Heterophyllus). *Jurnal Ilmiah Dr Aloei Saboe*, *8*(2), 1–10.

Chaudhry, G.-E.-S., Md Akim, A., Sung, Y. Y., & Sifzizul, T. M. T. (2022). Cancer and apoptosis: The apoptotic activity of plant and marine natural products and their potential as targeted cancer therapeutics. *Frontiers in Pharmacology*, *13*, 842376. https://doi.org/10.3389/fphar.2022.842376

Cheng, G. J., Leung, E. Y., & Singleton, D. C. (2022). In vitro breast cancer models for studying mechanisms of resistance to endocrine therapy. *Exploration of Targeted Anti-Tumor Therapy*, *3*(3), 297–320. https://doi.org/10.37349/etat.2022.00084

Cordoba, N., Fernandez-Alduenda, M., Moreno, F. L., & Ruiz, Y. (2020). Coffee extraction: A review of parameters and their influence on the physicochemical characteristics and flavour of coffee brews. *Trends in Food Science & Technology*, *96*, 45–60. https://doi.org/https://doi.org/10.1016/j.tifs.2019.12.004

Fadri, R. A., Sayuti, K., Nazir, N., & Suliansyah, I. (2020). Production Process and Quality Testing of Arabica Ground Coffee (Coffee arabica L) Solok Regency, West Sumatera. *Journal of Applied Agricultural Science and Technology*, *4*(1), 36–55. https://doi.org/10.32530/jaast.v4i1.135

Fatimah, F., Christin Larasati, N., Wicaksono, R., & Naufalin, R. (2019). Optimization of temperature and time of extraction of kecombrang stem and leaf (etlingera elatior) based on the quality of product bioactive components. *IOP Conference Series: Earth and Environmental Science*, *406*(1). https://doi.org/10.1088/1755-1315/406/1/012015

Felhi, S., A. Daoud, H. Hajlaoui, K. Mnafgui, N. Gharsallah, and A. Kadri. (2017). Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of Ecballium elaterium seeds and peels fruits. Food Science and Technology Campinas. Vol: 37(3): 483-492

Galán-Huerta, K. A., Rivas-Estilla, A. M., Martinez-Landeros, E. A., Arellanos-Soto, D., & Ramos-Jiménez, J. (2016). The Zika virus disease: An overview. *Medicina Universitaria*, *18*(71), 115–124. https://doi.org/10.1016/j.rmu.2016.05.003

https://doi.org/https://doi.org/10.1016/j.fshw.2017.06.001

Gurning, K., Simanjuntak, H. A., Purba, H., Situmorang, R. F. R., Barus, L., & Silaban, S. (2021). Determination of Total Tannins and Antibacterial Activities Ethanol Extraction Seri (Muntingia calabura L.) Leaves. *Journal of Physics: Conference Series*. https://doi.org/10.1088/1742-6596/1811/1/012121

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

Hasan, A.E.Z, Utami, N, F., & Mona. (2022). *Extraction Of Robusta Coffee Beans (Coffea Canephora) From Wonosobo By Ultrasonic Waves And Anticancer Tests*. *Fitofarmaka*. *12*(1), 89–99.

Hasan, A.E.Z, Andrianto, D., Rahmania, A., Ahmada, I. G. C., & Abdillah, R. M. (2021). Effect of Heating on Flavonoid Concentration in Honey Harvested From Across Indonesian Archipelago. *Pharmacologyonline*, *3*(615), 623.

Hutami, S. N., Triaminingsih, S., & Indrani, D. J. (2018). Effect of tooth immersion in the coffee drink with different types of coffee roast temperature on tooth discoloration. *Journal of Physics: Conference Series*, *1073*(3). https://doi.org/10.1088/1742-6596/1073/3/032026

Istiqomah, A., Muti'ah, R., & Hayati, E. K. (2015). Anticancer Activity against Breast Cancer Cells T47D and Identification of Its Compound from Extracts and Fractions of Leaves Bamboo Grass (Lophaterum gracile B.). *Alchemy*, *4*(1). https://doi.org/10.18860/al.v4i1.3138

Juarez, G. F. Y., Pabiloña, K. B. C., Manlangit, K. B. L., & Go, A. W. (2018). Direct Dilute Acid Hydrolysis of Spent Coffee Grounds: A New Approach in Sugar and Lipid Recovery. *Waste and Biomass Valorization*, *9*(2), 235–246. https://doi.org/10.1007/s12649-016-9813-9

Khobibah, Nurhidayati, T., Ruspita, M., Rozikhan, & Martanti, L. E. (2022). The Administration of Dutch Teak Leaf Tea Extract (Guazama Ulmifolia lamk) in

Reducing Cholesterol Levels in Depo Acceptors. *Jurnal Info Kesehatan*, *20*(2), 240–250. https://doi.org/10.31965/infokes.vol20.iss2.875

Kiyama, R. (2019). *Estrogenic Activity of Coffee Constituents*. Nutrients Review, *5092*, 1–20.

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

Latos-Brozio, M., & Masek, A. (2020). Natural Polymeric Compound Based on High Thermal Stability Catechin from Green Tea. *Biomolecules*, *10*(8). https://doi.org/10.3390/biom10081191

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

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

MinistryofHealth. (2013). *Suplemen III farmakope herbal Indonesia 2013 (Edisi 1)* (Vol. 2013, Issue Edisi 1). Kementerian Kesehatan R.I.

Muttaqin, Z., Lina Hadi, & Zara Maghfirah. (2022). Efficacy of Robusta Coffee Bean Extract (Coffea robusta) Against Bacterial Growth of Staphylococcus aureus. *Bioscientia Medicina : Journal of Biomedicine and Translational Research*, *6*(4), 1675–1679. https://doi.org/10.37275/bsm.v6i4.493

Polamuri, D., Valentina, C. G., Suresh, R., & Islam, A. (2020). In-vitro Anticancer and Antioxidant Activity of Green Coffee Beans Extract. *Asian Food Science Journal*, *17*(2), 24–35. https://doi.org/10.9734/afsj/2020/v17i230188

Pratama, M., Razak, R., & Rosalina, V. S. (2019). Analysis of Total Tannin Content of Clove Flower Ethanol Extract (Syzygium aromaticum L.) Using the Uv-Vis Spectrophotometric Method.. *Jurnal Fitofarmaka Indonesia*, *6*(2), 368–373. https://doi.org/10.33096/jffi.v6i2.510

Ramakrishna, A., Giridhar, P., Sankar, K. U., & Ravishankar, G. A. (2012). Melatonin and serotonin profiles in beans of Coffea species. *Journal of Pineal Research*, *52*(4), 470–476. https://doi.org/10.1111/j.1600-079X.2011.00964.x

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.

Shanmugam, S. (2015). Granulation techniques and technologies: recent progresses. *BioImpacts : BI*, *5*(1), 55–63. https://doi.org/10.15171/bi.2015.04

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

Sirait, P. S., Setyaningsih, I., & Tarman, K. (2019). Anticancer Activity of Spirulina Cultivated in Walne and Organic Media. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *22*(1), 50. https://doi.org/10.17844/jphpi.v22i1.25876

Utami, 2020. (2020). Potensi Antioksidan dari Biji Kopi Robusta 9 Daerah di Pulau Jawa. In *Lembaga Penelitian dan Pengabdian Pada Masyarakat Universitas Pakuan*.

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.

Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese Medicine (United Kingdom)*, *13*(1), 1–26. https://doi.org/10.1186/s13020-018-0177-x

Zhang, Z., Poojary, M. M., Choudhary, A., Rai, D. K., Lund, M. N., & Tiwari, B. K. (2021). Ultrasound processing of coffee silver skin, brewer's spent grain and potato peel wastes for phenolic compounds and amino acids: a comparative study. *Journal of Food Science and Technology*, *58*(6), 2273–2282. https://doi.org/10.1007/s13197-020-04738-2.