

Cytotoxic activity of water and ethanol extract of *Curcuma caesia* (black turmeric) on breast cancer cell line

^{1,2,*}Hairunisa, I., ³Da'I, M., ³Wikantyasning, E.R. and ¹Bakar, M.F.A.

¹Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (UTHM), 84600 Muar, Johor, Malaysia

²Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur (UMKT), Jl. Ir. H. Juanda No.15, Sidodadi, Kec. Samarinda Ulu, Kota Samarinda, Kalimantan Timur 75124, Indonesia

³Faculty of Pharmacy, Universitas Muhammadiyah Surakarta (UMS), Jalan Ahmad Yani, Pabelan, Kartasura, Surakarta 57162, Jawa Tengah, Indonesia

Article history:

Received: 5 December 2023

Received in revised form: 14

October 2024

Accepted: 26 October 2024

Available Online: 20

November 2024

Keywords:

Cytotoxic,
Black turmeric,
Curcuma caesia,
Breast cancer

DOI:

[https://doi.org/10.26656/fr.2017.8\(S5\).25](https://doi.org/10.26656/fr.2017.8(S5).25)

Abstract

Breast cancer is one of the cancers with the highest incidence and mortality rate in the world. *Curcuma caesia* (Black turmeric) is known to have neuroprotective, anthelmintic, sunscreen activity, anti-diabetic and also anti-cancer activity. This study aimed to determine the phytochemical content and possible cytotoxic activity of *C. caesia* water extract (EWCC) and *C. caesia* ethanol extract (EECC) on breast cancer cell lines. Samples were extracted using two extraction methods, maceration technique using 96% ethanol as solvent and double boiling using water as a solvent. The phytochemical content, total phenolic content (TPC), and total flavonoid content (TFC) of each extract were determined using spectrophotometer method. Cytotoxic activity was measured using MTT assay on MCF-7 and 4T1 breast cancer cell lines (24 hrs and 48 hrs). Results showed that EWCC and EECC contained flavonoids, alkaloids, and polyphenols. The values of TPC and TFC were 106.08 mg GAE/100 g and 4.32 mg QUE/g for EECC, respectively, and 73.29 mg GAE/100 g and 3.40 mg QUE/g for EECC, respectively. Meanwhile, the cytotoxic activity test found that EWCC did not provide cytotoxic activity on MCF-7 and 4T1 cells. In the MCF-7 cell line, EECC exhibited IC₅₀ values of 212.90 µg/mL at 24 hrs and 311.91 µg/mL at 48 hrs. Similarly, in the 4T1 cell line, EECC exhibited IC₅₀ values of 161.06 µg/mL at 24 hrs and 150.89 µg/mL at 48 hrs. From these results, it can be concluded that EECC has cytotoxic activity in MCF-7 and 4T1 breast cancer cell models, so this extract can be studied more deeply to determine its pathway of action.

1. Introduction

Cancer is a degenerative illness that impacts numerous individuals. Cancer is primarily induced by genetic mutations and is significantly associated with an individual's lifestyle (Anand *et al.*, 2008; Wishart, 2015). Breast cancer is one of the most prevalent cancers. Based on research conducted by Bray *et al.* (2018) the incidence of breast cancer in the world in 2018 reached 2.1 million cases. In the United States, the incidence of breast cancer is estimated to be approximately 250,000 cases (Ahmad, 2013). While in Malaysia and Indonesia, cases of breast cancer have reached 31% of cases of cancer in women (Tan *et al.*, 2018) and 16,7% of all cancer survivors and cause death until 11% (Baade, 2017). This statistic indicates that breast cancer is a prevalent type of cancer with a significant mortality rate.

Metastasis is the process by which cancer cells spread from the primary site to other organs that are distant from the primary site (Scully *et al.*, 2012). The incidence of Metastatic Breast Cancer (MBC) can be categorized as stage IV and is the main cause of death in breast cancer cases. MBC affects about 5% of breast cancer patients. The prognosis for MBC is known to be poor; recurrence is common, and MBC patients have a 5-year survival rate (Donovan, 2013). Furthermore, MBC therapy is only palliative, reducing symptoms, improving quality of life, extending survival time, and preventing cancer progression (O'Shaughnessy, 2005; Park *et al.*, 2022)

Chemotherapy, hormonal agents, and immunotherapy agents are all used in systemic treatment for breast cancer. The use of these three agents will

*Corresponding author.

Email: ih787@umkt.ac.id

initially produce positive results in 90% of breast cancer cases and 50% of metastases. (Gonzalez-Angulo *et al.*, 2007). Prolonged usage results in cancer resistance, thereby requiring the formulation of novel treatments to tackle this problem. Anticancer pharmaceuticals are synthesised through various methodologies, one of which involves the formulation of drugs derived from natural medicinal compounds. Utilising natural ingredients for treatment may serve as a less hazardous alternative with diminished side effects. Moreover, an alternative paradigm posits that natural ingredients can be utilised to prevent cancer and to augment cancer treatment with synthetic pharmaceuticals.

Plants from the *Zingiberaceae* family are plants that are known to have many beneficial activities such as antioxidants, antibacterial, anti-inflammatory, relieving pain, gastritis, dyspepsia, and anticancer (Chen *et al.*, 2008; Danciu *et al.*, 2015; Zahara *et al.*, 2018). Black turmeric (*Curcuma caesia*) is a plant of the *Zingiberaceae* family. This plant has antioxidant activity, anti-mutagen, smooth muscle relaxant activity, Haemorrhoids, leprosy, asthma, fever, menstrual disorder and anti-cancer (Devi *et al.*, 2015; Borah *et al.*, 2019).

The anticancer activity from ethanol extract of *C. caesia* showed direct cytotoxicity on the EAC cell line with IC₅₀ 90.7 mg/mL. Methanol extract of *C. caesia* exhibited a significant decrease in tumour volume, tumour weight, viable cell count and percentage increased the lifespan with a value 88.09% of EAC-treated mice (Karmakar *et al.*, 2013). Hexan extract of *C. caesia* also showed anti-proliferative effects on Hep2 (IC₅₀: 7.8 µg/mL), HepG2 (IC₅₀: 0.9765 µg/mL), HT29 (IC₅₀: 7.8125 µg/mL) and Vero (IC₅₀: 50 µg/mL). *Curcuma caesia* exerts an anti-proliferative effect by increasing the expression of pro-apoptotic proteins such as p53, Bax, Bim, caspase-9, and caspase-3 (Mukunthan *et al.*, 2017). Besides anti-cancer activity *C. caesia* also showed cancer chemoprevention activity, *C. caesia* showed chemopreventive abilities on hepatocellular carcinoma as indicated by the decrease in the concentration of carcinogenic markers such as AST, ALT, ALP, and AChE on diethylnitrosamine induced BABL/c mice (Hadem *et al.*, 2014). This plant also reduces genotoxicity and protects the liver and kidney in cyclophosphamide-induced Swiss albino mice (Devi and Mazumder, 2016). However, although many studies have been conducted on the anticancer and cancer chemoprevention activity of *C. caesia*, the anticancer activity of this plant on MCF-7 and 4T1 has never been explored.

The objective of this study was to investigate the

potential cytotoxic effects of *C. caesia* on MCF-7 and 4T1 breast cancer cell lines. MCF-7 is a human breast cancer cell line that is positive for estrogen receptors, while 4T1 serves as a model for high metastasis. The study will examine the composition of secondary metabolites, total phenolic content, and total flavonoid content in *C. caesia*. The assessments to be conducted encompass phytochemical screening and cytotoxicity evaluation utilising the MTT assay. This study aims to elucidate the potential of *C. caesia* as a natural therapeutic agent for breast cancer treatment.

2. Materials and methods

2.1 Materials

The rhizome of *Curcuma caesia*, 96% ethanol, Roswell Park Memorial Institute (RPMI) medium (Gibco), Dulbecco's Modified Eagle Medium (DMEM) high glucose medium (Gibco), MTT reagent (Merck), trypsin (Gibco), fetal bovine serum (Gibco), gallic acid PA (Merck), quercetin (Merck), Folin–Ciocalteu reagent (Merck), sodium carbonate, distilled water, micropipette (Dragonlab), UV-vis spectrophotometer (Genesys 10s), rotary evaporator (RV 10), vortex (B-One), elisa reader (Epoch), glassware (Iwaki), and freeze dryer was used in this study.

2.2 Sample determination

Sample determination was carried out at the Laboratory of Ecology and Conservation of Tropical Forest Biodiversity at the University of Mulawarman. The validity of the sample was confirmed by letter number 12/UNI7.4.08/LL/2021, which stated that the sample used was black turmeric (*Curcuma caesia*).

2.3 Sample preparation and extraction

The rhizome of *Curcuma caesia* was obtained from Johor, Malaysia. The sample was cleaned using a wet cloth. It was then cut into small pieces for further drying in an oven at 40°C. The dried sample was subsequently powdered using a blender and then sieved to obtain a sample with a size of less than 2 mm. The samples that were uniform in size were used for extraction. Extraction was carried out using two solvents, namely, 96% ethanol and distilled water. The first extraction used the maceration technique with 96% ethanol as a solvent for 3 days, and the extraction result was concentrated using a rotary evaporator (Bakar *et al.*, 2020). The second extraction employed a double boiling technique at 90°C for 15 mins, utilising distilled water as the solvent, and the resultant extracts were dehydrated using a freeze dryer.

2.4 Phytochemical analysis

Phytochemical analysis was conducted on the samples to determine the concentrations of secondary metabolites, including saponins, tannins, alkaloids, flavonoids, polyphenols, terpenoids, steroids, glycosides, and quinones. The phytochemical composition was assessed following standard protocols (Saifudin, 2014). Following the addition of specific reagents to the solution, tests were conducted through visual observation of colour change or precipitate formation.

2.5 Determination of total phenolic content

Total phenolic content (TPC) was determined using UV-Vis spectrophotometry at a wavelength of 765 nm (Yamin et al., 2021). Gallic acid with a concentration range of 3–50 µg/mL was used as standard. Samples were prepared by weighing 100 mg of samples and dissolving them with 1 mL 96% methanol (ethanol extract) and distilled water (water extract). Furthermore, up to 10 mL of 96% methanol was added in a volumetric flask. The samples were then vortexed for 10 mins and centrifuged to obtain the supernatant. Approximately 500 µL of sample and gallic acid solution (5 mg/mL) were pipetted and added with 0.5 mL of Folin–Ciocalteu reagent, shaken, and incubated for 8 mins. Next, 3 mL of 10% Na₂CO₃ solution and 10 mL of distilled water were added. The sample was then incubated for 2 hrs in a dark condition and read on a UV-vis spectrophotometer at a wavelength of 765 nm. Each sample was tested three times, and the results were expressed as mg gallic acid/100 g extract.

2.6 Determination of total flavonoid content

Total flavonoid content (TFC) was determined using UV-Vis spectrophotometry at a wavelength of 420 nm (Yamin et al., 2021). Quercetin with a concentration range of 5–180 µg/mL was used. Samples were prepared by weighing 100 mg of samples and dissolving them with 1 mL of 96% methanol (ethanol extract) and distilled water (water extract). Furthermore, up to 10 mL of 96% methanol was added to the volumetric flask. The samples were then vortexed for 10 mins and centrifuged to obtain the supernatant. Next, 750 µL of the sample supernatant and quercetin solution (5 mg/mL) was pipetted, and 750 µL of AlCl₃ solution (2%) was added. This mixture was then incubated for 1 hr in the dark, and the absorbance at a wavelength of 420 nm was read using methanol as a blank. Each sample was tested three times, and all the results were expressed as mg quercetin/g extract.

2.7 Cytotoxic activity using MTT Assay

The MTT assay was carried out based on Mosman

(1983) with modifications on MCF-7 and 4T1 breast cancer cell lines. Each cell (~2×10⁴ cells/well) was grown in 96 well plates. Cell cultures were incubated for 24 hrs in DMEM high glucose and RPMI medium and incubated at 37°C with a CO₂ level of 0.5%. Approximately 100 µL sample solution was put into the well, then incubated for 24 and 48 hrs. After that, cells were washed with 100 µL of PBS from each well, and then 5 mg/mL of MTT reagent was added to the culture medium, at each well. Incubate again for 2.5 hrs. After that, the media containing MTT was stopped by the addition of a stopper reagent (10% SDS in 0.01 N HCl). The plates were wrapped in aluminum foil and left overnight at room temperature. The absorbance was read using an ELISA reader with a wavelength of 595 nm. The absorbance data of a single treatment was converted into percent cell viability to calculate the IC₅₀ value.

3. Results and discussion

3.1 Sample extraction

Curcuma caesia (Black turmeric) is a plant that originated in India; however, it is also widely cultivated in China, Nepal, Malaysia, Thailand, and Indonesia nowadays (Kachura, 2019). In general, the outward appearance of *C. caesia* rhizome is not much different from that of other types of *Curcuma*. However, if this plant is cut crosswise, it shows a black circle in the middle of the rhizome (Figure 1). Traditionally, *C. caesia* is widely used for the treatment of various diseases. This plant is also used as the main ingredient for making health drinks. However, the prestige of *C. caesia* is still inferior and less explored than yellow turmeric (*Curcuma longa*).



Figure 1. The appearance of *Curcuma caesia* rhizome.

In this study, samples were extracted by maceration method using 96% ethanol (EECC) and double boiling technique using distilled water (EWCC) as a solvent. From the extraction process, the yields for EWCC and EECC were found to be 4.35% and 7.6%, respectively. Based on appearance, EWCC was light brown in colour with a nutty smell, while EECC was black and odorless (Table 1). Another study used water as a solvent but by using the maceration technique, and the yield was 3.13%. Meanwhile, the use of methanol via the

maceration technique gave a yield of 1.83% (Yadav and Saravanan, 2019). Another extraction process, which was carried out in other studies, used the soxhlet extraction technique, but the yield value of this extraction process could not be found (Pakkirisamy et al., 2014; Jose and Thomas, 2014; Ranemma and Reddy,

Table 1. Results of the extraction process from *Curcuma caesia*.

	<i>Curcuma caesia</i>	
	Ethanol	Water
Yield (%)	7.60	4.35
Organoleptic		
Odor	Odorless	Nutty
Color	Black	Light brown

2017).

3.2 Phytochemical analysis

A phytochemical screening test was conducted on EWCC and EECC to identify the types of secondary metabolites present in the samples. EWCC exhibited favorable outcomes for saponins, alkaloids, flavonoids, and polyphenols, whereas EECC demonstrated positive results for tannins, alkaloids, flavonoids, and polyphenols (Table 2). Other results of the aqueous extract of *C. caesia* from research conducted by Yadav et al. (2019) revealed that the aqueous and methanolic extracts of *C. caesia* contained secondary metabolites, such as flavonoids, saponins, carbohydrates, proteins and diterpenes (Abu Bakar et al., 2015; Yadav and Saravanan, 2019). Another discovery, utilising the soxhlet extraction method with methanol as a solvent, indicated that *C. caesia* also possessed alkaloids (Lawand and Gandhi, 2013). GC-MS analysis revealed that the methanol extract comprised various bioactive compounds, such as α -santalol, 9-cis-retinal, Ar-

Table 2. The results of phytochemical analysis on water extract and ethanol extract of *Curcuma caesia*.

Types of Secondary Metabolite	<i>Curcuma caesia</i>	
	Ethanol	Water
Saponin	-	+
Tannin	+	-
Steroid/Terpenoid	-	-
Alkaloid		
Mayer	+	+
Dragendorf	+	+
Buchardat	+	+
Flavonoids	+	+
Glycosides	-	-
Quinone	-	-
Polyphenol	+	+

(+): Indicates "Presence", (-): Indicates "Absence"

tumerone, and alloaromadendrone. The α -santalol was anticipated to exhibit cytotoxic activity on *C. caesia* (Pakkirisamy et al., 2014).

3.3 Determination of total phenolic content and total flavanoid content

Phenolic compounds and flavonoids are compounds that are closely related to the antioxidant activity of a plant (Abu Bakar et al., 2009; Aryal et al., 2019; Abuzaid et al., 2020). Consequently, a greater concentration of phenolic and flavonoid compounds is anticipated to correlate with enhanced antioxidant activity. Compounds rich in antioxidants are thought to inhibit the development of cancer in individuals. This results from antioxidants' capacity to inhibit damage and genetic mutations induced by free radicals. The test results indicated that the TPC values for EWCC and EECC were 106.08 and 73.29 mg GAE/100 g, respectively. Simultaneously, the TFC values for EWCC

Table 3. The results of total phenolic content and total flavanoid content on aqueous extract and ethanol extract of *Curcuma caesia*.

	<i>Curcuma caesia</i>	
	Ethanol	Water
Total Phenolic Content (mg GAE/100 g)	73.29	106.08
Total Flavonoid Content (mg QUE/g sample)	3.40	4.32

and EECC were 4.32 and 3.40 mg QUE/g, respectively (Table 3).

3.4 Cytotoxic activity using MTT Assay

Cytotoxic tests were performed on EECC and EWCC samples in MCF-7 and 4T1 breast cancer cell models. MCF-7 cells were used as a model of estrogen receptor-positive human breast cancer cell line, whereas 4T1 is a highly metastatic model of breast cancer cell line. Breast cancer cell models are divided into estrogen receptor positive and estrogen receptor negative. Where it is known, the type of estrogen positive breast cancer cell model is a cancer cell model that is still responsive to treatment. While the estrogen negative breast cancer cell model is a breast cancer cell model that is not responsive to treatment and leads to the occurrence of metastases (Amin et al., 2022). Cytotoxic activity test was performed within 24 hrs and 48 hrs with the results expressed as Inhibitory Concentration 50% (IC₅₀) on each breast cancer cell model used.

The treatment with EWCC samples for 24 hrs revealed that EWCC had no cytotoxic activity in both MCF-7 and 4T1 cells (Figure 2 and Figure 3). The graph indicates that EWCC exhibited no cytotoxic activity until

the maximum concentration was applied, and the viability of MCF-7 and 4T1 cells remained between 98-100%. The EWCC cytotoxic activity test was not conducted for 48 hrs.

The EECC demonstrated cytotoxic activity in the MCF-7 and 4T1 breast cancer cell models, in contrast to EWCC samples. This is indicated by a decrease in cell viability as sample concentration increases (Figure 2 and Figure 3). The IC₅₀ values of EECC in MCF-7 cancer cells for treatment times of 24 hrs and 48 hrs were 212.90 ug/mL and 311.91 ug/mL, respectively.

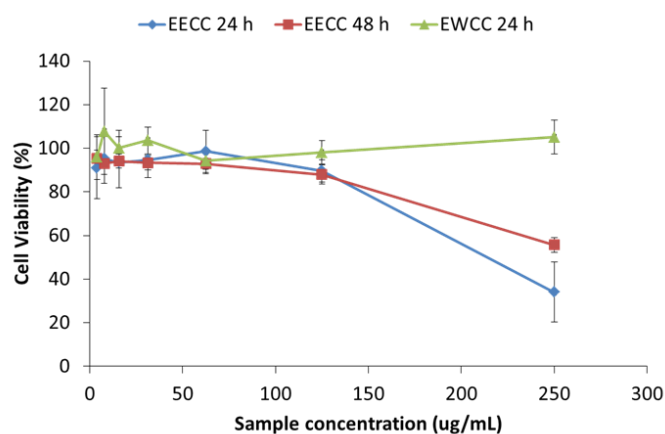


Figure 2. The cytotoxic activity result of EWCC and EECC on MCF-7 breast cancer cell line.

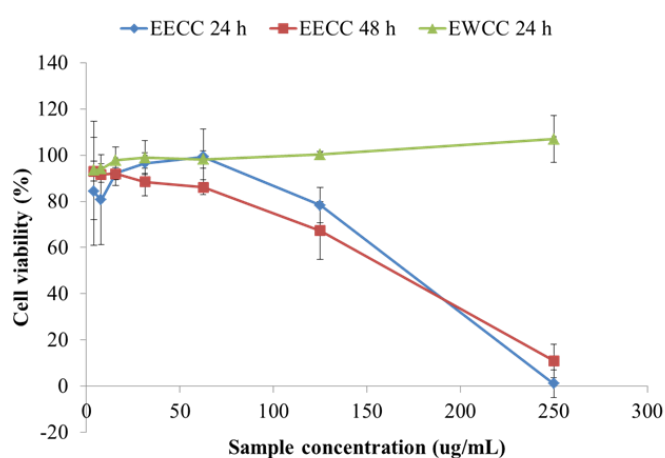


Figure 3. The cytotoxic activity result of EWCC and EECC on 4T1 breast cancer cell line.

Meanwhile, after 24 and 48 hrs in 4T1 cancer cells, the IC₅₀ concentrations were 161.06 ug/mL and 150.89 ug/mL, respectively.

Curcuma caesia's cytotoxic activity has received little attention. We were able to obtain only a few publications concerning its cytotoxic activity. According to several published studies, *C. caesia* has anti-cancer, anti-metastatic, and cancer chemoprevention properties. The anticancer activity of *C. caesia* extract has been tested on EAC cell lines both *in vitro* and *in vivo*. Secondary metabolites of phenols, tannins, terpenoids, saponins, alkaloids, flavonoids, and volatile oils are

thought to be responsible for those anticancer activities (Karmakar *et al.*, 2013). *Curcuma caesia* hexan extract also demonstrated anticancer activity against Hep2, HepG2, HT, and Vero cell lines by increasing the expression of pro-apoptotic proteins such as p53, Bax, Bim, caspase-9, and caspase-3 (Bakar *et al.*, 2010; Mukunthan *et al.*, 2017). This activity is also supported by the *in silico* approach using Auto Dock Vina on some proteins that involve Mis-Match Repair (MMR) such as *abl1*, *myc*, *max*, *myb*, *pca*, *top3a*, *p73*, and *blm* on Haematologic cancer. The *in silico* study discovered three compounds that were suspected of providing this activity, but the names of these compounds were not mentioned (Bhattacharya and Patel, 2021). *Curcuma caesia* has antimetastatic activity in addition to anticancer activity. The antimetastatic activity of *C. caesia* chloroform and hexane fractions was demonstrated by inhibiting MDA-MB-231 movement by 2%, 36%, and 54% at concentrations of 50, 100, and 150 uM, respectively. When compared to the positive control, the value of this inhibition is quite high. Curcuzederon, a molecule thought to provide antimetastatic activity, was also mentioned in this study (Al-Amin *et al.*, 2021).

Surprisingly, our findings showed that EECC had higher cytotoxic activity in non-responsive and highly metastatic 4T1 breast cancer cells than MCF-7. This implies that EECC has good activity in breast cancer cell models that should be resistant to treatment. As a result, we suspect that EECC has anti-metastatic activity, though this needs to be investigated further. Furthermore, we suspected that there was a link between antioxidant activity and cytotoxic activity at first. In this study, the total phenol and flavonoid results showed that EWCC had a higher total phenol and flavonoid value than EECC. However, EECC demonstrated greater cytotoxic activity than EWCC. Then, we discovered that total phenolic and antioxidant activity had no relationship with cytotoxic activity. (Osman *et al.*, 2020). Indeed, cancer mortality is closely related to an increase in intracellular reactive oxygen species (ROS) (Yang *et al.*, 2018; Perillo *et al.*, 2020), implying that substances with cytotoxic activity must be pro-oxidants rather than antioxidants. A plant's antioxidant activity is more focused on cancer prevention rather than cancer treatment.

Subsequent to the study's findings, EECC exhibits anticancer potential due to its comparatively elevated IC₅₀ value, especially in highly metastatic 4T1 breast cancer cells. Consequently, forthcoming research may focus on the anti-metastatic properties of EECC and its underlying mechanisms of action. The mechanisms of cytotoxic activity are predominantly associated with the

apoptotic induction pathway.

4. Conclusion

According to the findings of this study, EWCC had no cytotoxic activity in the MCF-7 and 4T1 breast cancer cell models. Meanwhile, EECC showed cytotoxic activity in MCF-7 and 4T1 cells, with IC₅₀ values of 212.90 g/mL (24 hrs) and 311.91 g/mL (48 hrs), respectively, as well as 161.06 g/mL (24 hrs) and 150.89 g/mL (48 hrs). The EECC's cytotoxic activity was greater in non-responsive and highly metastatic 4T1 breast cancer cells than in MCF-7. Consequently, subsequent investigations may concentrate on the anti-metastatic characteristics of EECC and its cytotoxic mechanism, particularly within the 4T1 cell line.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The author would like to acknowledge the funding of the study by Matching RE-SIP grant with code number M069 entitled “*In vitro* anti-cancer potential of black halia (*Kaempferia parviflora*) against breast cancer cell lines”.

References

- Abu Bakar, M. F., Ahmad, N. E., Suleiman, M., Rahmat, A. and Isha, A. (2015). Garcinia dulcis Fruit Extract Induced Cytotoxicity and Apoptosis in HepG2 Liver Cancer Cell Line. *BioMed Research International*, 2015, 916902. <https://doi.org/10.1155/2015/916902>
- Abu Bakar, M. F., Mohamed, M., Rahmat, A. and Fry, J. (2009). Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus*). *Food Chemistry*, 113(2), 479–483. <https://doi.org/10.1016/j.foodchem.2008.07.081>
- Abuzaid, H., Amin, E., Moawad, A., Usama Ramadan, Abdelmohsen, Hetta, M. and Mohammed, R. (2020). Liquid Chromatography High-Resolution Mass Spectrometry Analysis, Phytochemical and Biological Study of Two Aizoaceae Plants: A New Kaempferol Derivative from *Trianthema portulacastrum* L. *Pharmacognosy Research*, 12, 212-218.
- Al-Amin, M., Eltayeb, N.M., Khairuddean, M. and Salhimi, S.M. (2021). Bioactive chemical constituents from *Curcuma caesia* Roxb. rhizomes and inhibitory effect of curcuzederone on the migration of triple-negative breast cancer cell line MDA-MB-231. *Natural Product Research*, 35(18), 3166–3170. <https://doi.org/10.1080/14786419.2019.1690489>
- Amin, I.M., Ruslan, N.B., Zulkipli, Z.A., Zakaria, N.A., Jalil, M.T.M. and Aris, F. (2022). Cytotoxic Effect of Dillapiole on Human Breast Cancer MCF-7 Cells. *Malaysian Applied Biology*, 51(4), 29–35. <https://doi.org/10.55230/mabjournal.v51i4.08>
- Anand, P., Kunnumakara, A.B., Sundaram, C., Harikumar, K.B., Tharakan, S.T., Lai, O.S., Sung, B. and Aggarwal, B. B. (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharmaceutical Research*, 25, 2097-2116. <https://doi.org/10.1007/s11095-008-9661-9>
- Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R. and Koirala, N. (2019). Total Phenolic content, Flavonoid content and antioxidant potential of wild vegetables from western Nepal. *Plants*, 8(4), 96. <https://doi.org/10.3390/plants8040096>
- Baade, P. (2017). Geographical variation in breast cancer outcomes. *International Journal of Environmental Research and Public Health*, 14(5), 523. <https://doi.org/10.3390/ijerph14050523>
- Bakar, F.I.A., Bakar, M.F.A., Abdullah, N., Endrini, S. and Fatmawati, S. (2020). Optimization of Extraction Conditions of Phytochemical Compounds and Anti-Gout Activity of *Euphorbia hirta* L. (Ara Tanah) Using Response Surface Methodology and Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis. *Evidence-Based Complementary and Alternative Medicine*, 2020, 4501261. <https://doi.org/10.1155/2020/4501261>
- Bakar, M.F.A., Mohamed, M., Rahmat, A., Burr, S.A. and Fry, J.R. (2010). Cytotoxicity and polyphenol diversity in selected parts of *Mangifera pajang* and *Artocarpus odoratissimus* fruits. *Nutrition and Food Science*, 40(1), 29–38. <https://doi.org/10.1108/00346651011015890>
- Bhattacharya, P. and Patel, T.N. (2021). A study of deregulated MMR pathways and anticancer potential of curcuma derivatives using computational approach. *Scientific Reports*, 11, 10110. <https://doi.org/10.1038/s41598-021-89282-5>
- Borah, A., Paw, M., Gogoi, R., Loying, R., Sarma, N., Munda, S., Kumar Pandey, S. and Lal, M. (2019). Chemical composition, antioxidant, anti-inflammatory, anti-microbial and in-vitro cytotoxic efficacy of essential oil of *Curcuma caesia* Roxb. leaves: An endangered medicinal plant of North East India. *Industrial Crops and Products*, 129, 448-454. <https://doi.org/10.1016/j.indcrop.2018.12.035>

- Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A. and 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/10.3322/caac.21492>
- Ahmad, A. (Ed.) (2013). *Breast Cancer Metastasis and Drug Resistance*. New York, USA: Springer. <https://doi.org/10.1007/978-1-4614-5647-6>
- Chen, I.N., Chang, C.C., Ng, C.C., Wang, C.Y., Shyu, Y.T. and Chang, T.L. (2008). Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. *Plant Foods for Human Nutrition*, 63, 15-20. <https://doi.org/10.1007/s11130-007-0063-7>
- Danciu, C., Vlaia, L., Fetea, F., Hancianu, M., Coricovac, D.E., Ciurlea, S.A., Şoica, C.M., Marincu, I., Vlaia, V., Dehelean, C.A. and Trandafirescu, C. (2015). Evaluation of phenolic profile, antioxidant and anticancer potential of two main representants of Zingiberaceae family against B164A5 murine melanoma cells. *Biological Research*, 48, 1. <https://doi.org/10.1186/0717-6287-48-1>
- Devi, H. and Mazumder, P. (2016). Methanolic Extract of *Curcuma caesia* Roxb. prevents the toxicity caused by Cyclophosphamide to bone marrow cells, liver and kidney of mice. *Pharmacognosy Research*, 8(1), 43-49. <https://doi.org/10.4103/0974-8490.171106>
- Devi, H.P., Mazumder, P.B. and Devi, L.P. (2015). Antioxidant and antimutagenic activity of *Curcuma caesia* Roxb. rhizome extracts. *Toxicology Reports*. <https://doi.org/10.1016/j.toxrep.2014.12.018>
- Donovan, D. (2013). Metastatic breast cancer epidemiology and management with a focus on taxanes. *Clinical Journal of Oncology Nursing*, 17, 5-9. <https://doi.org/10.1188/13.CJON.S1.5-8>
- Gonzalez-Angulo, A. M., Morales-Vasquez, F. and Hortobagyi, G.N. (2007). Overview of resistance to systemic therapy in patients with breast cancer. In Yu, D. and Hung, M.C. (Eds.) *Breast Cancer Chemosensitivity. Advances in Experimental Medicine and Biology*, Vol. 608. New York, USA: Springer. https://doi.org/10.1007/978-0-387-74039-3_1
- Hadem, K.L.H., Sharan, R.N. and Kma, L. (2014). Inhibitory potential of methanolic extracts of *Aristolochia tagala* and *Curcuma caesia* on hepatocellular carcinoma induced by diethylnitrosamine in BALB/c mice. *Journal of Carcinogenesis*, 13, 7. <https://doi.org/10.4103/1477-3163.133520>
- Jose, S. and Thomas, T.D. (2014). Comparative phytochemical and anti-bacterial studies of two indigenous medicinal plants *Curcuma caesia* Roxb. and *Curcuma aeruginosa* Roxb. *International Journal of Green Pharmacy*, 8(1), 65-71. <https://doi.org/10.4103/0973-8258.126828>
- Karmakar, I., Dolai, N., Suresh Kumar, R.B., Kar, B., Roy, S.N. and Haldar, P.K. (2013). Antitumor activity and antioxidant property of *Curcuma caesia* against Ehrlich's ascites carcinoma bearing mice. *Pharmaceutical Biology*, 51(6), 753-759. <https://doi.org/10.3109/13880209.2013.764538>
- Lawand, R.V. and Gandhi, S.V. (2013). Comparison of *Curcuma caesia* Roxb. with other Commonly Used Curcuma Species by HPTLC. *Journal of Pharmacognosy and Phytochemistry*, 2(4), 126-131.
- Mukunthan, K.S., Satyan, R.S. and Patel, T.N. (2017). Pharmacological evaluation of phytochemicals from South Indian Black Turmeric (*Curcuma caesia* Roxb.) to target cancer apoptosis. *Journal of Ethnopharmacology*, 209, 82-90. <https://doi.org/10.1016/j.jep.2017.07.021>
- O'Shaughnessy, J. (2005). Extending Survival with Chemotherapy in Metastatic Breast Cancer. *The Oncologist*, 10(S3), 20-29. <https://doi.org/10.1634/theoncologist.10-90003-20>
- Osman, M.A., Mahmoud, G.I. and Shoman, S.S. (2020). Correlation between total phenols content, antioxidant power and cytotoxicity. *Biointerface Research in Applied Chemistry*, 11(3), 10640-10653. <https://doi.org/10.33263/BRIAC113.1064010653>
- Pakkirisamy, M., Kalakandan, S.K. and Ravichandran, K. (2014). Phytochemical screening, GC-MS, FT-IR analysis of methanolic extract of *Curcuma caesia* roxb (black turmeric). *Pharmacognosy Journal*, 9(6), 952-956. <https://doi.org/10.5530/pj.2017.6.149>
- Park, M., Kim, D., Ko, S., Kim, A., Mo, K. and Yoon, H. (2022). Breast Cancer Metastasis: Mechanisms and Therapeutic Implications. *International Journal of Molecular Sciences*, 23(12), 6806. <https://doi.org/10.3390/ijms23126806>
- Perillo, B., Di Donato, M., Pezone, A., Di Zazzo, E., Giovannelli, P., Galasso, G., Castoria, G. and Migliaccio, A. (2020). ROS in cancer therapy: the bright side of the moon. *Experimental and Molecular Medicine*, 52(2), 192-203. <https://doi.org/10.1038/s12276-020-0384-2>
- Ranemma, M. and Reddy, S.K. (2017). Phytochemical Investigation Study of *Curcuma caesia* Roxb Different Geographical Regions (Delhi and Orissa) of India. *IOSR Journal of Biotechnology and*

- Biochemistry*, 3(1), 23–26. <https://doi.org/10.9790/264x-0301012326>
- Rani, V.S. (2018). *In Vitro* Cytotoxic Activity and Preliminary Phytochemical Analysis of the Crude Extracts of *Eleutherine bulbosa* (Miller), Urban. *World Journal of Pharmaceutical Research*, 7(4), 1022–1029. <https://doi.org/10.1039/c6ra17788c>
- Saifudin, A. (2014). *Senyawa Alam Metabolit Sekunder: Teori, Konsep dan Teknik Pemurnian*. Yogyakarta, Indonesia: Yogyakarta Deepublish. [In Bahasa Indonesia].
- Scully, O.J., Bay, B.H., Yip, G. and Yu, Y. (2012). Breast cancer metastasis. In *Cancer Genomics and Proteomics*, 9(5), 311-320.
- Tan, M.-M., Ho, W.-K., Yoon, S.-Y., Mariapun, S., Hasan, S.N., Lee, D.S.-C., Hassan, T., Lee, S.-Y., Phuah, S.-Y., Sivanandan, K., Ng, P.P.-S., Rajaram, N., Jaganathan, M., Jamaris, S., Islam, T., Rahmat, K., Fadzli, F., Vijayanathan, A., Rajadurai, P., See, M.-H., Thong, M.-K., Mohd Tiab, N.A., Yip, C.-H. and Teo, S.H. (2018). A case-control study of breast cancer risk factors in 7,663 women in Malaysia. *PLoS ONE*, 13(9), e0203469. <https://doi.org/10.1371/journal.pone.0203469>
- Wishart, D.S. (2015). Is Cancer a Genetic Disease or a Metabolic Disease? *eBioMedicine*, 2(6), 478-479. <https://doi.org/10.1016/j.ebiom.2015.05.022>
- Yadav, M. and Saravanan, K.K. (2019). Phytochemical Analysis and Antioxidant Potential of Rhizome Extracts of *Curcuma amada* Roxb and *Curcuma caesia* Roxb. *Journal of Drug Delivery and Therapeutics*, 9(5), 123–126. <https://doi.org/10.22270/jddt.v9i5.3609>
- Yamin, Ruslin, Mistriyani, Sabarudin, Ihsan, S., Armadany, F.I., Sahumena, M.H. and Fatimah, W.O.N. (2021). Determination of total phenolic and flavonoid contents of Jackfruit peel and in vitro antiradical test. *Food Research*, 5(1), 84–90. [https://doi.org/10.26656/fr.2017.5\(1\).350](https://doi.org/10.26656/fr.2017.5(1).350)
- Yang, H., Villani, R.M., Wang, H., Simpson, M.J., Roberts, M.S., Tang, M. and Liang, X. (2018). The role of cellular reactive oxygen species in cancer chemotherapy. *Journal of Experimental and Clinical Cancer Research*, 37, 266. <https://doi.org/10.1186/s13046-018-0909-x>
- Zahara, M., Hasanah, M. and Zalianda, R. (2018). Identification of Zingiberaceae as medicinal plants in Gunung Cut Village, Aceh Barat Daya, Indonesia. *Journal of Tropical Horticulture*, 1, 24-28. <https://doi.org/10.33089/jthort.v1i1.9>