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Total Phenolic Content and Antioxidant, Toxicity, and Anticancer Against MCF-7 Breast Cancer Cell Lines of Indonesian *Curcuma* Rhizomes Extracts

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Abstract:
Background: *Curcuma* plants (Zingiberaceae) have been widely used by Asians as a treatment for various diseases. Phytochemical studies show that Curcuma has bioactivity as antioxidant, antibacterial, antifungal, anticancer, antiviral, and anti-inflammatory. This research aims to compare the total phenolic content and antioxidant, toxicity, and anticancer against MCF-7 breast cancer cell lines activities of five *Curcuma* plants grown in Bogor, Indonesia.
Materials and Methods: Plants used in this research are the rhizomes of temu kunci (*C. rotunda*), temu putih (*C. zedoaria*), temu mangga (*C. mangga*), temu giring (*C. heyneana*), and temu hitam (*C. aeruginosa*). All plants were macerated using acetone, ethanol, and methanol. Total phenolic content from all plant extracts was determined using Folin-Ciocalteu analysis. Moreover, antioxidant activity was measured following DPPH assay, toxicity was determined using the BSLT method with *Artemia salina* shrimp larvae, while anticancer activity was analyzed by MTT assay against MCF-7 breast cancer cell lines.
Results: Acetone extract of temu kunci (TKEA) had the highest total phenolic content (72.52 mg GAE/100 g). TKEA was the most active extract as an antioxidant (IC_{50} = 205.41 ppm). TKEA has the highest toxicity against A. salina larvae (LC_{50} = 16 ppm). Ethanol extract of temu giring (TGEE) was the most active extract to inhibit MCF-7 cells proliferation (IC_{50} = 0.65 ppm).
Conclusion: The antioxidant, toxicity activities, and anticancer activities of TGEE reveal that compounds contained in TGEE are a potential candidate for further development of breast cancer treatment, while antioxidant activities and mechanisms of compounds contained in TGEE need to be learned using various antioxidant assays.
Key Word: Anticancer, Antioxidant, Curcuma, Toxicity, Phenolic value.

I. INTRODUCTION

Zingiberaceae family is one of the most widespread plant groups in the world. Around 51 genera and 1200 species of plants have been identified. Most Zingiberaceae plants can be found in tropical forests[1]. Asians have used *Curcuma,* a family of Zingiberaceae plants as a food spice and traditional medicine to cure fever, cough with phlegm, rheumatism, asthma, to purify blood, liver, indigestion, nausea, diarrhea, and skin diseases. In particular, Indonesians use *Curcuma* plants for the treatment of diabetes, high blood pressure, and cancer. Various studies to test the biological activity of *Curcuma* have been carried out through the total phenol content, antioxidant activity, toxicity, antibacterial, antifungal, anticancer, antiviral, and anti-inflammation tests[2].

Phenolic is one of the secondary metabolites produced by plants. Phenolic compounds have antioxidant activities. An antioxidant compound can reduce the reactivity of free radical compounds and stop the chain reaction that can destroy macromolecules in the body[3]. Antioxidant compounds reduce the potential of cell mutation and the formation of cancer cells by reducing the reactivity of radical compounds[4]. Various studies have shown that methanol, ethanol, and chloroform extracts of *Curcuma mangga* (temu mangga), *C. aeruginosa* (temu hitam), *C. zedoaria* (temu putih), *C. rotunda* (temu kunci), and *C. heyneana* (temu giring) planted in several places have antioxidant activities[3–6]. Methanol, dichloromethane, acetone, ethanol, *n*-hexane, chloroform, and ethyl acetate extracts of those five *Curcuma* plants have anticancer activity against cancer cells such as MCF-7, HL-60, HepG2, DU-145, NCI-H460, MDA, MB 231, HBL-100, PC3, HT-29, and Ca Ski[6–11].

The pharmacological effect of *Curcuma* plants is strongly influenced by the content of secondary metabolites in the plants. Different growth areas, growth conditions, soil types, cultivation environments, and harvest times of plants can affect the metabolites composition and amount in the plant. The extraction process and solvent can also affect the pharmacological effects of plants. Plants' pharmacological effects can be tested using a toxicity assay. The plants have a pharmacological effect if it's toxic. Toxicity assay can be carried out using Brine Shrimp Lethality Test (BSLT) method[12]. In line with the information about compounds composition and bioactivity of *Curcuma* plants, this study aims to compare the total phenolic content and antioxidant, toxicity, and anticancer against MCF-7 breast cancer cell lines activities of five *Curcuma* plants.

II. MATERIAL AND METHODS

Materials

The rhizomes of *C. mangga*, *C. heyneana*, *C. zedoaria*, *C. rotunda*, and *C. aeruginosa* were obtained from Trop BRC garden LPPM-IPB, West Java, Indonesia in January 2019. Chemicals used for extraction were from technical (CV Firman) grades, i.e. acetone, ethanol, and methanol. Chemicals used for analysis were Tween 80, dimethyl sulphoxide (DMSO), Folin-Ciocalteu reagent, NaOH, gallic acid, DPPH reagent, ascorbic acid, RPMI media, MCF-7 cell, shrimp *Artemia salina* larvae, and seawater.

Sample extraction

Fresh plant's rhizomes were cleaned by water, sliced into small pieces, dried, then grounded by a powdering mill into a fine powder. The dried plants' powders were macerated using ethanol, acetone, and methanol three times at room temperature. The ratio of sample and solvent is 1:5. The plant extracts then evaporated by a rotary evaporator to produce the viscous and solvent-free extract.

Total phenolic content

The total phenolic content of the plant was quantified using Folin-Ciocalteu method. The sample dissolved into the ethanol at concentration 500 ppm, then diluted to 250, 125, 62.5, 31.25, and 15.625 ppm. The sample solution was taken 1 mL into the tube, then added by 5 mL reagent Folin-Ciocalteu 7.5%. The solution was mixed and standstill for 8 minutes, then added by 4 mL NaOH 1%. The solution was standstill for 1 hour. The absorbance of the solution was quantified at 739 nm. Gallic acid (5-100 ppm) was used to create a calibration curve and determine samples' total phenolic content. Total phenolic content in the extracts showed in milligram equivalent to gallic acid/100 gram sample (mg EAG/100 g sample)[13].

Antioxidant activity

Antioxidant activity was measured using the DPPH method. The extract dissolved into ethanol at concentration 500 ppm, then diluted to 250, 125, 62.5, 31.25, and 15.625 ppm. The sample solution was taken 500 µL from each extract concentration, then added by 500 µL DPPH solute (125 µM in ethanol). The mixture was homogenized and incubated into the darkroom at room temperature for 30 minutes. The absorbance of the mixture was measured at 517 nm using spectrophotometer UV-Vis DU 7500. Ascorbic acid was used as the positive control. The antioxidant power against DPPH calculated using the equation:

$$\text{Inhibition (\%)} = \frac{(A-B)}{A} \times 100$$

A is blank absorbance (mixture of methanol and DPPH) and B is the sample absorbance (mixture of extract and DPPH). IC_{50} value calculated based on the curve correlation between sample concentration and inhibition percentage[14].

Brine shrimp lethality test

The extract dissolved at a concentration of 500 ppm. The extract was taken 0.025 g was mixed with 0.1 mL Tween 80 solution, then added by seawater until it reached 25 mL. Extract solution diluted into 200 ppm by took 10 mL of solution at concentration 500 ppm then add the seawater until 50 mL. As many as 10 shrimp *A. salina* larvae were added into the vial using quantified seawater. Gradual dilution was done to the extract concentration 200 ppm into the vial contained shrimp larvae until the concentration 10, 25, 50, 75, and 100 ppm of the solution was obtained. Observation and quantification of the dead shrimp larvae were done after 24 hours of incubation. Percentage of shrimp larvae's death calculated and converted into probit score, then LC_{50} score calculated from the regression curve of correlation between concentration logarithm and probit score[15,16].

$$\text{Dead Larvae (\%)} = \frac{Dead\ larvae\ (sample - blank)}{total\ of\ the\ tested\ larvae} \times 100\%$$

Anticancer activity

Anticancer activity was tested using MTT assay to MCF-7 breast cancer cells. The cells were grew using concentration 5000 cells in 100 µL growth media. The growth media used in this assay was RPMI 1640. The extract was added after the cell reach confluent 50% or after 24 hours. The MTT reagent 5 mg/mL was taken 10 µL into the wells, then incubated in 4 hours at 37℃. The formazan crystal formed in the incubation process dissolved into ethanol and the absorbance was measured at 595 nm[17].

III. RESULT

Figure 1 shows extraction yield percentage of *Curcuma* plants using various organic solvents. The extraction yield percentage range of temu mangga (TM), temu kunci (TK), temu hitam (TH), temu putih (TP), and temu giring (TG) using ethanol (E), methanol (M), and acetone (A) are various. TKEE has the largest extraction yield percentage, while THEA has the smallest extraction yield percentage.

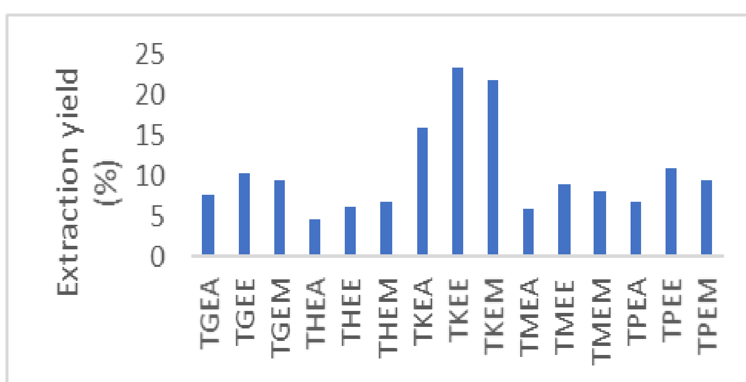

Figure 1: Extraction yield percentage of *Curcuma* plants with various organic solvents.

Figure 2shows total phenolic content (TPC) of *Curcuma* plants. The TPC score is amount of gallic acid (EAG) per 100 gram samples. TPEA had the lowest TPC and TKEA had the highest TPC with 11.22 and 72.52 mg EAG/100 g samples, respectively.

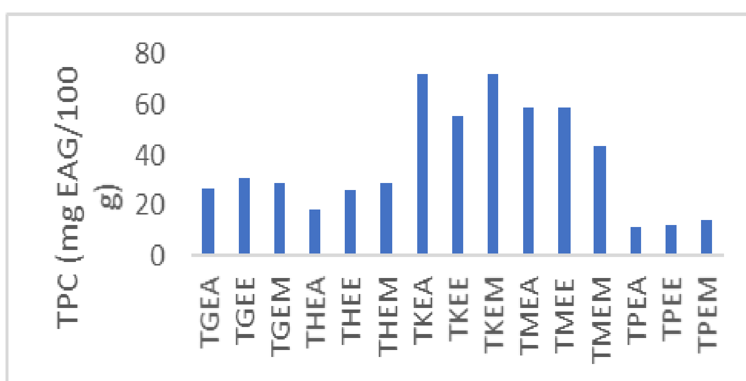

Figure 2 : Total phenolic content of *Curcuma*plants

Figure 3 shows the IC_{50} of all *Curcuma* plant Extracts on antioxidant assay. The IC_{50} of all plant extracts is higher than 200 ppm.

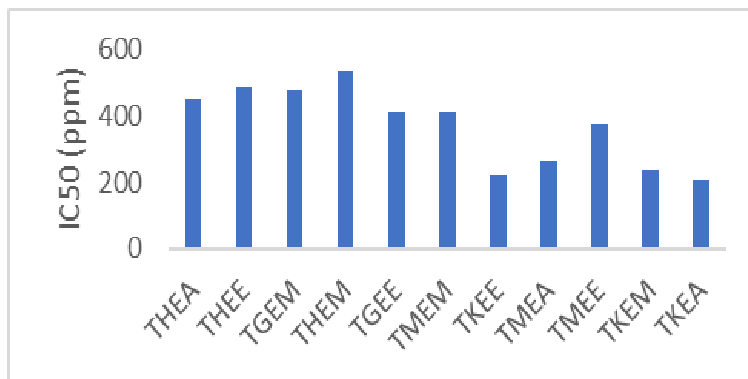

Figure 3 : IC_{50} of *Curcuma* extracts on antioxidant assay

Figure 4 shows the result of toxicity assay of *Curcuma* extracts using BLST method towards *A. salina* larvae.

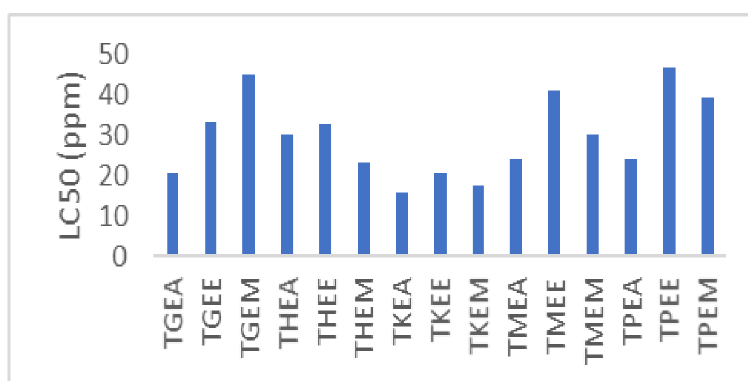

Figure 4: Toxicity of *Curcuma* extracts based on BSLT assay

Figure 5 shows the IC_{50} of *Curcuma* plant extracts toward MCF-7 cells' proliferation. Epirubicin was the positive control in this assay and its IC_{50} was 0.52 ppm.

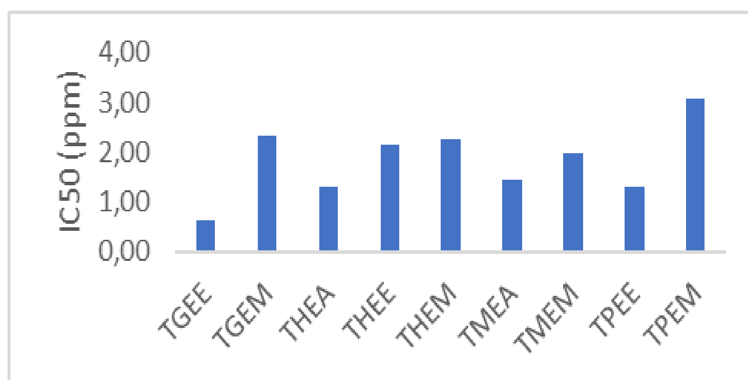

Figure 5 : IC_{50} of *Curcuma*'s extracts toward MCF-7 cancer cells' proliferation

Figure 6 shows the physical appearance of MCF-7 cells before (a) and after the addition (b) of plant extracts. Extract plant used in the *in vivo* analysis was TKEA 62.5 ppm. The alive cell has a dark color, while the dead cell was colorless.

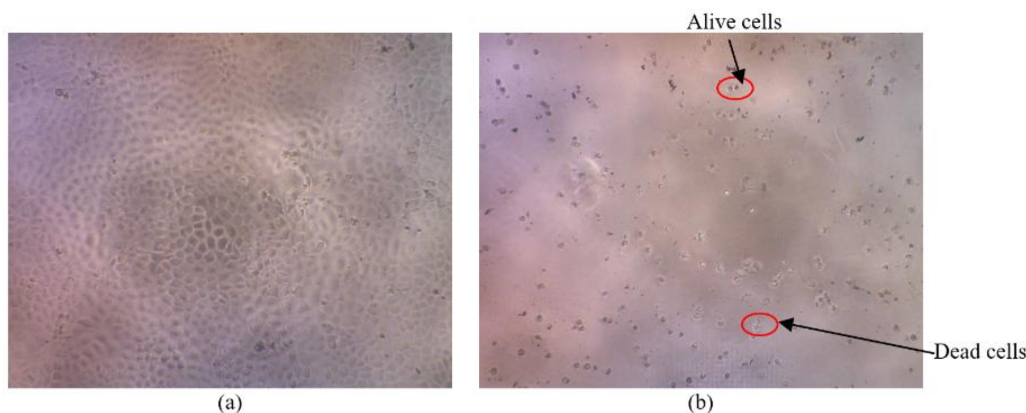

Figure 6: Physical apperance of MCF-7 cells control (a) and after addition of TKEA 62.5 ppm (b)

IV. DISCUSSION

Extraction of all plant samples using organic solvents such as methanol (M), ethanol (E), and acetone (A). The dielectric constant of methanol, ethanol and acetone at 25℃ are 32.5, 23.55, and 19.10, respectively[18]. The dielectric constant of organic solvent is directly proportional to its increasing in polarity[19], so solvent order from the most polar to the slight polar solvent are methanol, ethanol, and acetone. The increasing polarity of the solvent will increase the extract's yield percentage produced by the extraction process because the non-polar until polar compounds will dissolve into the solvent[20].

The ethanolic extracts from almost all plants have the highest yield percentage, followed by methanol and acetone, respectively (Figure 1). The yield results are different from the previous study[20], whereas ideally, methanol extract has the highest yield percentage, followed by ethanol and acetone, respectively. This difference showed that ethanol is better than acetone and methanol to extract the compounds in the plants. TK had the highest yield percentage from overall samples, while TH had the lowest yield percentage with an average yield of 20.42% and 5.88%, respectively. Order from the highest to lowest yield percentage is TK, TP, TG, TM, and TH. Based on the extraction yield percentage, there are many polar compounds in TK and non-polar compounds in TP. TK and TM had the average TPC thrice and twice bigger than TP. TPC concentrations in the plants from the largest were TK, TM, TG, TH, and TP (Figure 2).

Antioxidant activity was assessed using the DPPH method because of its advantages, such as simple, easy, fast, sensitive, reproducible, and need a small amount of sample. The working principle of this method is the donor of the hydrogen atom from antioxidant compound to radical DPPH and the color-changing of DPPH from violet to bright yellow. The change of color intensity will decrease the absorbance of DPPH at its maximal wavelength[21]. The antioxidant power of a compound or extract is divided into four categories based on its IC_{50}, i.e. very strong (<50 ppm), strong (50-100 ppm), moderate (100-150 ppm), and low (150-200 ppm)[22].

TK had the best antioxidant activities among all plants and its average IC_{50} is 222 ppm (Figure 3). The order of plants from the strongest antioxidant activity to the lowest is TK, TM, TG, TH, and TP. The measured result of *Curcuma*'s antioxidant activity is different from the previous studies. The IC_{50} of THEE based on the measured result and previous studies were 461.51, 681.39, and 406,52 ± 0,02 ppm, respectively[2,23]. The IC_{50} of THEM based on the measured result and previous study were 533.82 and 199.71, respectively[3]. The IC_{50} of TKEE based on measure result and previous study were 223.22 and 389,051 ± 0,426 ppm, respectively[24]. The IC_{50} of TGEM based on the measured result and previous studies were 479.84, 155.68, and >500 ppm, respectively[3,25]. The IC_{50} of TGEE based on the measured result and previous study were 413.53 and 60.08 ± 1.17 ppm, respectively[25]. The IC_{50} of TMEM based on the measured result and previous study were 411.21 and 90.42 ppm, respectively[3]. The IC_{50} of TMEE based on the measured result and previous study were 376.01 and 277.79 ppm, respectively[26]. The difference of all IC_{50} results were indicated the difference of compounds contained in the extracts and antioxidant work mechanisms toward DPPH.

BSLT assay is a preliminary assay used to detect the toxicity of extract or compound. BSLT assay was chosen because it is easy to use, fast, low cost, and accurate[27]. Toxicity measuring based on *A. salina*'s death percentage. The larvaes' death percentage is proportional to the sample's toxicity towards cancer cells[28]. Toxicity of the sample showed as LC_{50}, the concentration needed to kill 50% of the animal tested's population. There are three toxicity categories based on its concentration, i.e. very toxic (LC_{50}< 30 μg/mL), toxic (LC_{50} = 30–1000 μg/mL), and nontoxic (LC_{50}> 1000 μg/mL)[29].

All extracts were toxic towards *A. salina* shrimp larvae (Figure 4). TGEA, THEM, TKEA, TKEE, TKEM, TMEA, and TPEA were very toxic. TKEA had the highest toxicity, while TPEA had the lowest toxicity. The result also showed that all extracts of TK are toxic toward *A. salina* larvae. The comparison of

LC_{50} based on the measured result and the previous report showed in Table 1. There is only a little information about toxicity based on the BSLT assay of those five *Curcuma* plants. There is no information about the BSLT assay and LC_{50} data of TM, TK, and TG plant extracts. The difference LC_{50} results of THEM and TPEE based on measured results and kinds of the literature indicated that there are some different compounds in the plants.

Table no 1: LC_{50} score by BSLT assay for *Curcuma* plants based on literature and measured result

Plant	Data Source	Sample (Extract, compound)	Plant's Part	LC_{50} (ppm)
Temu Hitam (*C. aeruginosa*)	Previous study[36]	Methanol	Rhizome	107.5
	Previous study[37]	Ethanol	Rhizome	37,7
	Measurement result	Methanol	Rhizome	23,4
		Ethanol	Rhizome	33
		Acetone	Rhizome	30,1
Temu mangga (*C. mangga*)	Previous study[38]	Methanol	Leaves	6,5
	Measurement result	Methanol	Rhizome	30,3
		Ethanol	Rhizome	41,1
		Acetone	Rhizome	24,3
Temu putih (*C. zedoaria*)	Previous study[39]	Ethanol	Rhizome	22,9
	Measurement result	Methanol	Rhizome	39,4
		Ethanol	Rhizome	46,8
		Acetone	Rhizome	24,3
Temu kunci (*B. rotunda*)	Previous study[40]	Pinocembrin	Rhizome	23,3
		Pinostrobin	Rhizome	60,5
	Measurement result	Methanol	Rhizome	17,7
		Ethanol	Rhizome	20,6
		Acetone	Rhizome	16,0
Temu Giring (*C. heyneana*)	Measurement result	Methanol	Rhizome	45,1
		Ethanol	Rhizome	33,2
		Acetone	Rhizome	20,6

MTT assay works principle is measuring the inhibition power of extract toward MCF-7 cells proliferation. The inhibitory power of extract towards MCF-7 cells' proliferation was quantified using an inhibition 50% (IC_{50}) score. The extract works as an inhibitor towards MCF-7 cells' proliferation if its $IC_{50} \leq 30$ μg/mL. The inhibitory power of sample classified into very active ($IC_{50} \leq 5$ μg/mL), active ($IC_{50} = 5$–10 μg/mL), and moderate ($IC_{50} = 11$–30 μg/mL)[30].

TKEE, TPEA, TGEA, TMEE, TKEM, and TKEA were nonactive as an inhibitor of MCF-7 cells' proliferation (Figure 5). Based on the grouping and comparison using the positive control, TGEE, TPEE, THEA, TMEA, TMEM, THEE, THEM, TGEM, and TPEM were very active to inhibit the proliferation of MCF-7 cells. Acetone, ethanolic, and methanolic extracts of TK were inactive as an inhibitor. Previous studies showed that Panduratin A and Boesenbergin A isolated from TK were very active ($IC_{50} = 3.75$ ppm) and moderate ($IC_{50} = 25.43 \pm 0.36$ ppm), respectively[31,32]. The presence of Panduratin A and Boesenbergin A showed that TK still could inhibit MCF-7 cells' proliferation, but there may be an interaction of the compounds in the extract that reduce the inhibitory ability of TK.

TKEA had the highest inhibitory ability ($IC_{50} = 1.16$ ppm) among all extracts. TKEA at 62.5 ppm had the highest inhibitory percentage (95.87%) based on MTT assay (Figure 6). MCF-7 cells are round, oval, or irregular in two-dimension appearance and round in three-dimension[33]. The cell was shrunk after the addition of TKEA. The shrunk cell happened because of reduction of cytoplasm volume, shrinkage of the nucleus, and merge of membrane and cell's organelles[34]. The death of MCF-7 cells also marked by the reduction of violet color after the addition of MTT reagent into the cells. The alive cells will cut off the tetrazolium ring at the MTT reagent and produce the violet formazan crystal[35].

Inhibitory power of *Curcuma* plants from measurement results and previous studies compared and showed in Table 2. The measured IC_{50} scores were smaller than the previous studies. The IC_{50} of TGEM both measured results and a previous study showed that TGEM was inactive to inhibit MCF-7 cells' proliferation[10]. The difference in inhibition ability of extracts is caused by the different metabolites produced by plants and their cultivation processes. Zerumin B, *(E)*-labda-8(17),12-diene-15,16-dial, and Panduratin A were very active to inhibit MCF- 7 cells' proliferation and their IC_{50} were 0.59 ± 1.8 ppm, 4.3 ± 1.30 ppm, dan 3.75 ppm, respectively[7,31].

Pearson correlation test was used to know the correlation between two variables. Correlation between TPC and antioxidant, antioxidant and toxicity, antioxidant and inhibition of cells' proliferation, and toxicity and inhibition of cells' proliferation could be studied using the Pearson correlation test. The correlation produced by this test was positive and negative. Positive and negative correlations were trusted if the p-value < 0.05. A positive correlation of analysis between TPC and antioxidant showed that phenolic compounds in the extract have antioxidant activity and increasing of antioxidant activity was in line with the TPC. Pearson correlation test

showed that there was a significant negative in TG and TP with r = -1.0000. Correlation between TPC and antioxidant activity of TH, TM, and TK had p-value > 0.05. A negative correlation in all extracts of TG and TP indicated that other secondary metabolites had antioxidant activity. Phenolic and terpenoid compounds such as curcumin, demethoxycurcumin, bis-demethoxycurcumin, and 5-isopropylidene-3,8-dimethyl-1(5H)-azulenone had antioxidant activities[41,42].

Pearson correlation test showed a significant positive correlation between antioxidant activity and toxicity in TG (r = 1.0000). The amount of antioxidant compounds in TG is in line with the toxicity of the plant. Non-phenolic compounds indicated had toxicity toward A. salina shrimp larvae. Pearson correlation test of TM extracts showed no correlation between TPC and antioxidant activity but showed a positive correlation between antioxidant activity and inhibition of MCF-7 cells' proliferation. Based on the Pearson correlation test, TM had non-phenolic compounds. There was a possibility that the non-phenolic compounds of TM were antioxidant agents and can inhibit MCF-7 cells' proliferation. There was no correlation between toxicity and inhibition of MCF-7 cells' proliferation. This comparison marked that there was a possibility of different inhibition mechanisms or cells destroying by TM.

BSLT and MTT assays are common tests to know the sample's potency as an anticancer. BSLT is the first assay to evaluate the hidden potency of extract as an antiproliferation agent, then continue using MTT assay to discover inhibition power of sample towards cancer cell[43]. So, the result of the BSLT assay is commonly in line with the MTT assay result. Tests of the samples showed that TPEA, TGEA, TMEE, TKEE, TKEM, and TKEA were toxic but could not inhibit MCF-7 cells' proliferation. Moreover, the TPC of all extracts was too various. A general comparison could not be used to show that phenolic compounds were the cause of the anticancer effect in the extracts. Through the information carried out by the tests, it can be assumed that there was a toxic compound with a poor inhibition mechanism to MCF-7 cells using MTT assay. A previous study reported that flavonoid and terpenoid in plant extract were toxic and had activity as antioxidant and anticancer[44].

TPEE, TPEM, THEA, THEE, TGEM, THEM, TGEE, and TMEM were inactive as an antioxidant but toxic and can inhibit MCF-7 cells' proliferation. This result was contradictive with the information which told that an antioxidant compound also had an anticancer activity[45]. The antioxidant assay was tested using the DPPH method. DPPH method was effective to quantify an antioxidant activity from hydroxyl-contained compounds substituted at para or orto position by -OH or -O-alkyl[46]. Hydroxyl-contained compounds are generally known as phenolic and flavonoid compounds. Based on the comparison of the information, it can be informed that there was a group compound with an unquantified antioxidant activity using DPPH method but was toxic and can inhibit MCF-7 cells' proliferation. Antioxidant assays can be tested using several methods such as CUPRAC, ABTS, and FRAP to know the antioxidant mechanism of the samples.

Table 2: Comparison of IC_{50} from MTT assay to MCF-7 cell proliferation using methanolic extract of *Curcuma*

Plant	IC_{50} (ppm)	Character	Data Source
Temu Hitam (*C. aeruginosa*)	2,26	Very Strong	Measurement Result
	>100	Nonactive	Previous study[6]
Temu mangga (*C. mangga*)	1,98	Very Strong	Measurement Result
	27,9 ± 0,3	Moderate	Previous study[9]
Temu putih (*C. zedoaria*)	3,09	Very Strong	Measurement Result
	>100	Nonactive	Previous study[11]
	21,0 ± 1,6	Moderate	Previous study[47]
Temu kunci (*B. rotunda*)	17,7	Moderate	Measurement Result
	51	Nonactive	Previous study[48]
Temu Giring (*C. heyneana*)	45,1	Nonactive	Measurement Result
	61,63 ± 1,76	Nonactive	Previous study[10]

V. CONCLUSION

Plants' extracts contained various concentrations of phenolic compounds. Acetone extract of temu kunci (TKEA) had total phenolic content 72.52 mg EAG/100 g and it was the highest total phenolic content among all extracts. TKEA was the most active and toxic extract with IC_{50} and LC_{50} score 205.41 ppm and LC_{50} 16 ppm, respectively. Ethanolic extract of temu giring (TGEE) was the most active extract to inhibit MCF-7 cells' proliferation with IC_{50} score of 0.65 ppm. The tests that have been carried out showed that TGEE and TKEA were the potential extracts to be used as anticancer and antioxidants. Further research and development need to be done regarding the anticancer and antioxidant bioactivities of those extracts.

REFERENCES

[1]. Kress WJ, Prince LM, Williams KJ. The phylogeny and a new classification of the gingers (zingiberaceae): evidence from 5moleculardata. American Journal of Botany. 2002;89(10):1682-1696.

[2]. Sandrasari DA, Sabariman M, Azni IN. Determination of potential level of indonesian rhizomes as an antioxidant based on phenolic compound and antioxidant activity. IOP Conference Series Earth and Environmental Science. 2019;383(1):1-6.

[3]. Jalip IS, Suprihatin, Wiryanti I, Sinaga E. Antioxidant activity and total flavonoids content of *Curcuma*rhizome extract. The 4 Th International Conference Green Technology Maulana Malik Ibrahim State Islamic University. 2014;93-99.

[4]. Samarasinghe B, Kaliyadasa E, Marasinghe P. Investigation of bioactivities and physicochemical properties of four different *Curcuma*species available in sri lanka. Journal of Medicinal Plants and By-Products. 2021;1:83-88.

[5]. Zulaikha N. Chemical Constituents of *Curcuma heyneana* and *Curcuma zedoaria*. 2017.

[6]. Atun S, Arianingrum R, Aznam N, Malek SNA. Isolation of sesquiterpenes lactone from *Curcuma aeruginosa*rhizome and the cytotoxic activity against human cancer cell lines. International Journal of Pharmacognosy and Phytochemical Research. 2016;8(7):1168-1172.

[7]. Abas F, Lajis NH, Israf DA, Khozirah S, Kalsom YU. Antioxidant and nitric oxide inhibition activities of selected malay traditional vegetables. Food Chemistry. 2006;95(4):566-573.

[8]. Sundram TCM, Zakaria MHB, Nasir MHBM. Antioxidant and cytotoxic effects of *Curcuma mangga* and *Bosenbergia rotunda*ethanolic extracts on MCF-7 cancer cell lines. Science Heritage Journal. 2019;3(2):10-14.

[9]. Malek SNA, Lee GS, Hong SL, Yaacob H, Wahab NA, Weber JFF, Shah SAA. Phytochemical and cytotoxic investigations of *Curcuma mangga*rhizomes. Molecules. 2011;16(6):4539-4548.

[10]. Atun S, Nurfina A, Arianingrum R, Nurestri S. Efek sitotoksik ekstrak umbi tumbuhan temu giring (*Curcuma heyneana*) dan temu ireng (*Curcuma aeruginosa*) terhadap beberapa sel kanker. Jurnal Penelitian Saintek. 2010;15(2):1-9.

[11]. Hamdi OAA, Rahman SNSA, Awang K, et al. Cytotoxic constituents from the rhizomes of *Curcuma zedoaria*. Scientific World Journal. 2014;2014:321943.

[12]. Suhirman S, Hernani, Syukur C. Uji toksisitas ekstrak lempuyang gajah (*Zingiber zerumbet*) terhadap larva udang (*Artemia salina* Leach.). Buletin Penelitian Tanaman Rempah dan Obat. 2015;17(1):30-38.

[13]. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju YS. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. Journal of Food and Drug Analysis. 2014;22(3):296-302.

[14]. Salazar-Aranda R, Pérez-López LA, López-Arroyo J, Alanís-Garza BA, de Torres NW. Antimicrobial and antioxidant activities of plants from northeast of mexico. Evidence-Based Complementary and Alternative Medicine. 2011;2011:536139.

[15]. Zuraida. Analisis toksisitas beberapa tumbuhan hutan dengan metode *brine shrimp lethality test* (BSLT). Jurnal Penelitian Hasil Hutan. 2018;36(3):239-246.

[16]. Rohmah RN, Ratnaningtyas NI, Asnani A. Kajian toksisitas dari tubuh buah *Ganoderma lucidum* dengan metode *brine shrimp lethality test* (BST). Scripta Biologica. 2014;1(1):30-32.

[17]. Haryanti S, Widiyastuti Y. Aktivitas sitotoksik pada sel MCF-7 dari tumbuhan indonesia untuk pengobatan tradisional kanker payudara. Media Penelitian dan Pengembangan Kesehatan. 2017;27(4):247-254.

[18]. Åkerlöf G. dielectric constants of some organic solvent-water mixtures at various temperatures. Journal of the American Chemical Society. 1932;54(11):4125-4139.

[19]. Khan MF, Rashid RB, Mian MY, Rahman MS, Rashid MA. Effects of solvent polarity on solvation free energy, dipole moment, polarizability, hyperpolarizability and molecular properties of metronidazole. Bangladesh Pharmaceutical Jorunal. 2016;19(1):9-14.

[20]. Firdiyani F, Agustini TW, Ma'ruf WF. Ekstraksi senyawa bioaktif sebagai antioksidan alami *Spirulina platensis*segar dengan pelarut yang berbeda. Jurnal Pengolahan Hasil Perikanan Indonesia. 2015;18(1):28-37.

[21]. Molyneux P. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin Journal of Science and Technology. 2004;26(2):211-219.

[22]. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199-1200.

[23]. Nurcholis W, Khumaida N, Syukur M, Bintang M, Ardyani IDAAC. Phytochemical screening, antioxidant and cytotoxic activities in extracts of different rhizome parts from *Curcuma aeruginosa* Roxb. International Journal of Research in Ayurveda and Pharmacy. 2015;6(5):634-637.

[24]. Atun S, Handayani S, Rakhmawati A. Potential bioactive compounds isolated from *Boesenbergia rotunda* as antioxidant and antimicrobial agents. Pharmacognosy Journal. 2018;10(3):513-518.

[25]. Kusumawati I, Oky KK, Rullyansyah S, Prijo TA, Widyowati R, Ekowati J, Hestianah EP, Maat S, Matsunami K. Anti-aging properties of *Curcuma heyneana* Valeton & Zipj: ascientific approach to its

use in javanese tradition. Journal of Ethnopharmacology. 2018;225:64-70.
[26]. Widowati W, Mozef T, Risdian C, Ratnawati H, Tjahjani S, Sandra F. The comparison of antioxidative and proliferation inhibitor properties of *Piper betle* L., *Catharanthus roseus* [L] G.Don, *Dendrophtoe petandra* L., *Curcuma mangga* Val. extracts on T47Dcancer cell line. International Research Journal of Biochemistry and Bioinformatics. 2011;1(2):22-28.
[27]. Tavakoli S, Vatandoost H, Zeidabadinezhad R, Hajiaghaee R, Hadjiakhoondi A, Abai MR, Yassa N. Gas chromatography, gc/mass analysis and bioactivity of essential oil from aerial parts of *Ferulago trifida*: antimicrobial, antioxidant, AChE inhibitory, general toxicity, mttassay and larvicidal activities. Journal of Arthropod-Borne Diseases. 2017;11(3):414-426.
[28]. Colegate SM, Molyneux RJ. Bioactive natural products: detection, isolation, and structural determination 2nd edition. CRC Press, Florida. 2008.
[29]. Yani DF, Sugita P, Syahbirin G. Phytochemicals and cytotoxicity of sausage fruit (*Kigelia africana*) extract against breast cancer cells MCF-7 in vitro. Journal of Pharmacy Research. 2018;12(3):288-292.
[30]. Cao S, Sng VHL, Wu XH, Sim KY, Tan BHK, Pereira JT, Goh SH. Novel cytotoxic polyprenylated xanthonoids from *Garcinia gaudichaudii* (guttiferae). Tetrahedron. 1998;54(36):10915-10924.
[31]. Kirana C, Jones GP, Record IR, McIntosh GH. Anticancer properties of panduratin aisolated from *Boesenbergia pandurata* (zingiberaceae). Journal of Natural Medicines. 2007;61(2):131-137.
[32]. Ng KB, Bustamam A, Sukari MA, Abdelwahab SI, Mohan S, Buckle MJC, Kamalidehghan B, Nadzri NM, Anasamy T, hadi AHA, Rahman HS. Induction of selective cytotoxicity and apoptosis in human t4-lymphoblastoid cell line (CEMss) by boesenbergin aisolated from *Boesenbergia rotunda*rhizomes involves mitochondrial pathway, activation of caspase 3 and g2/mphase cell cycle arrest. BMC Complementary and Alternative Medicine. 2013;13(41):1-15.
[33]. Wang H, Qian J, Zhang Y, Xu W, Xiao J, Suo A. Growth of mcf-7 breast cancer cells and efficacy of anti-angiogenic agents in a hydroxyethyl chitosan/glycidyl methacrylate hydrogel. Cancer Cell International. 2017;17(1):1-14.
[34]. Ghobrial IM, Witzig TE, Adjei AA. Targeting apoptosis pathways in cancer therapy. Cancer Prevention Research. 2015;55:178-194.
[35]. Haryoto, Muhtadi, Indrayudha P, Azizah T, Suhendi A. Aktivitas sitotoksik ekstrak etanol tumbuhan sala (*Cynometra ramiflora* Linn) terhadap sel HeLa, T47D dan WiDR. Jurnal Penelitian Saintek. 2013;18(2):21-28.
[36]. Mustariani BAA, Izuddin A, Hasyim DM, Batubara I. Uji toksisitas dan aktivitas antimakan ekstrak rimpang temu hitam (*Curcuma Aeruginosa* Roxb). Jurnal Farmasetis. 2017;6(1):1-8.
[37]. Zulfiah, Megawati, Herman, Lau SHA, Hasyim MF, Murniati, Roosevelt A, Kadang Y, Izza N, Patandung G. Uji toksisitas rimpang temu hitam (*Curcuma aeruginosa* Roxb.) terhadap larva udang (*Artemia salina* Leach) dengan metode *brine shrimp lethality test* (BSLT). Jurnal Farmasi Sandi Karsa. 2020;6(1):44-49.
[38]. Hossain MS, Rahaman MM, Rahman MA, Uddin J, Ahmed F. Phytochemical and biological investigation of *Curcuma amada*leaves. Dhaka University Journaln of Pharmaceutical Sciences. 2020;19(1):9-13.
[39]. Sumantri AW. Uji aktivitas sitotoksik dari tumbuhan temu putih (*Curcuma zedoaria*) asal kabupaten ogan komering ulu. Journal of Chemical Information and Modeling. 2020;53(9):1689-1699.
[40]. Tanjung M, Tjahjandarie TS, Sentosa MH. Antioxidant and cytotoxic agent from the rhizomes of *Kaempferia pandurata*. Asian Pacific Journal of Tropical Disease. 2013;3(5):401-404.
[41]. Pujimulyani D, Wazyka A, Anggrahini S, Santoso U. Antioxidative properties of white saffron extract (*Curcuma mangga* Val) in the β-carotene bleaching and DPPH-radical scavenging methods. Indonesian Food and Nutrition Progress. 2014;11(2):35-40.
[42]. Mau JL, Lai EYC, Wang NP, Chen CC, Chang CH, Chyau CC. Composition and antioxidant activity of the essential oil from *Curcuma zedoaria*. Food Chemistry. 2003;82(4):583-591.
[43]. Kumar PS, Febriyanti RM, Sofyan FF, Luftimas DE, Abdulah R. Anticancer potential of *Syzygium aromaticum* L. in MCF-7 human breast cancer cell lines. Pharmacognosy Research. 2014;6(4):350-354.
[44]. Elsyana V, Bintang M, Priosoeryanto BP. Cytotoxicity and antiproliferative activity assay of clove mistletoe (*Dendrophthoe pentandra* (L.) Miq.) leaves extracts. Advances in Pharmacological Sciences. 2016;3242698:1-6.
[45]. Musdalipah, Tee SA, Karmilah, Sahidin, Fristiohady A, Yodha AWM. Total phenolic and flavonoid content, antioxidant, and toxicity test with BSLT of *Meistera chinensis*fruit fraction from southeast sulawesi. Borneo Journal of Pharmacy. 2021;4(1):6-15.
[46]. Marjoni MR, Afrinaldi, Novita AD. Total content of fenol and antioxidant activity of the aqueous extract of cherry leaf (*Muntingia calabura* L.). Jurnal Kedokteran Yarsi. 2015;23(3):187-196.
[47]. Rahman SNSA, Wahab NA, Malek SNA. In vitro morphological assessment of apoptosis induced by

antiproliferative constituents from the rhizomes of *Curcuma zedoaria*. Evidence-Based Complementary and Alternative Medicine. 2013;257108:1-15.

[48]. Jing LJ, Mohamed M, Rahmat A, Bakar MFA. Phytochemiscals, antioxidant properties and anticancer investigations of the different parts of several gingers species (*Boesenbergia rotunda*, *Boesenbergia pulchella* var *attenuata* and *Boesenbergia armeniaca*). Journal of Medicinal Plants Research. 2010;4(1):27-32.

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