THE EXTRACTION OF CURCUMINOIDS FROM ETHANOL EXTRACT OF YELLOW TURMERIC (*Curcuma longa* L) AND ACTIVITY TEST ON P-388 MURINE LEUKEMIA CELLS

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Received : September 4, 2021 Accepted : April 21, 2022 Published : June 30, 2022

Abstract: Research on the Extraction of Curcuminoids from Ethanol Extract of Yellow Turmeric (*Curcuma longa* L) and its Activity Test on P-388 Murine Leukemia Cells has been carried out. This study aims to determine the per cent yield, solvent concentration, activity of P-388 murine leukemia cells and pH of the number of curcuminoids produced in ethanol extract. The curcuminoids were obtained by the extraction process through the soxhlet method using 70 and 96% ethanol solvent with a ratio of sample to solvent of 1:8 with variations in operating time for 3, 6, 9 and 12 hours. The absorbance of curcuminoid extract was measured to determine the concentration of curcumin. The cytotoxic test on murine leukemia P-388 cells used the Microculture Tetrazolium Technique (MTT) method. The results obtained were yellow turmeric curcuminoid compounds using 96% ethanol solvent at an extraction operating time of 9 hours. The largest extract yield was 21.15% with a concentration of 1121.10 ppm, which has activity against murine leukemia P-388 cells with an IC_{50} of 6.15 g/mL have the potential as anticancer leukemia, and dye analysis at acidic pH (2,4,6) is yellow, while alkaline pH (9,12) is brownish red as a natural food coloring.
Keywords: curcuminoids; dyes; extraction; yellow turmeric

Abstrak: Penelitian ekstraksi kurkuminoid dari ekstrak etanol kunyit kuning (*Curcuma longa* L) dan uji aktivitasnya terhadap sel murine leukimia P-388 telah dilakukan. Penelitian ini bertujuan untuk mengetahui rendemen, konsentrasi pelarut, aktivitas sel murine leukemia P-388 dan pH terhadap jumlah kurkuminoid yang dihasilkan pada ekstrak etanol. Kurkuminoid diperoleh dengan proses ekstraksi dengan metode sokletasi menggunakan pelarut etanol 70 dan 96% dengan perbandingan sampel dan pelarut 1:8 variasi waktu operasi selama 3, 6, 9 dan 12 jam. Ekstrak kurkuminoid diukur absorbansi untuk mengetahui konsentrasi kurkumin. Pengujian sitotoksik terhadap sel murine leukimia P-388 dengan metode *Microculture Tetrazolium Technique* (MTT). Hasil yang diperoleh senyawa kurkuminoid kunyit kuning menggunakan pelarut etanol 96% pada waktu operasi ekstraksi 9 jam rendemen ekstrak terbesar 21,15% dengan konsentrasi 1121,10 ppm, memiliki aktivitas terhadap sel murine leukimia P-388 dengan IC_{50} sebesar 6,15 µg/mL berpotensi sebagai antikanker leukemia, dan analisis zat warna pada pH asam (2,4,6) berwarna kuning, pada pH basa (9,12) berwarna merah kecoklatan sebagai pewarna alami makananan.
Kata Kunci: kurkuminoid; zat warna; ekstraksi; kunyit kuning

Recommended APA Citation :

Wati, I., Pratiwi, V.D., & Musadi, M.R. (2022). The Extraction of Curcuminoids From Ethanol Extract of Yellow Turmeric (*Curcuma longa* L) and Activity Test on P-388 Murine Leukemia Cells. *Elkawnie*, *8*(1). 65-77. https://doi.org/10.22373/ekw.v8i1.10720

Introduction

It is estimated that Indonesia has 30,000 out of 40,000 kinds of plants that exist in the world. About 9,600 is reported to be medicinal plants and 300 of them have been used in traditional medicine (Fazil *et al*., 2017). One of them is yellow turmeric (*Curcuma longa* L*)*, which belongs to the Zingiberaceae family that is quite popular and has been traditionally cultivated in Asian countries (Prabowo *et al*., 2019). The main chemical compound in yellow turmeric is curcuminoid whose 2.5-6% of it consists of curcumin as the basic compound, its derivatives desmethoxycurcumin and bisdemethoxycurcumin that have hydroxyl group, ketone group, and methoxy (Mutiah, 2015; Sahne *et al*., 2016).

Curcuminoid is a part of a compound group in the genus *Curcuma* that can be used as a natural coloring for food and traditional medicines (Sa'diyah *et al.,* 2015). Curcumin 2-5% of the curcuminoid is used in traditional medicines that are beneficial as anti-inflammation, antioxidant, anticancer, and chemotherapy (Seca 2018). Besides that, the curcuminoid compound has the potential to be developed as a natural coloring for food. Natural coloring from curcuminoid tends to be unstable because of its pH function, so some buffer solution is needed to make the color stable (Koswara, 2009).

Cancer is a degenerative disease that becomes the cause of death number two in the world after heart attacks. According to the data from International Union Against Cancer (IUAC), a cancer organization in the United States, in 2018, there are 12.4 million new cases of cancer, 7.6 million deaths from cancer, and 25 million people living with cancer. One of the kinds of cancer that can cause death is leukemia. Leukemia or blood cancer is a kind of cancer that affects the bone marrow and lymph tissue (Rusanti *et al.,* 2017). The treatment of cancer especially leukemia is by doing radiation and chemotherapy. Those kinds of treatments have several unwanted side effects (Sa'diyah *et al*., 2015). To anticipate the side effects and the expensive cost of the treatment, the search for the natural medication in yellow turmeric that has the potential to be anticancer needs to keep being done.

Some research disclosed the results of the extraction, some of which was the extraction of yellow turmeric with acetone solvent using soxhlet method resulted in yield extract of 22.8% Revathy *et al*., 2011), yellow turmeric with acetone solvent and yield extract of 69.67% (Popuri, 2013), Indian bay leaves with ethanol solvent, n-hexane and water with the highest yield extract of water of 11.4% (Azis *et al.* 2014), yellow turmeric with methanol solution: yield extract of water of 10.84% (Verma 2014), yellow turmeric essential oil with destilation and soxhlet

methods using ethanol solvent 100% having antioxidant activity with value of IC_{50}, 44.17 ± 0.04 µg/ml (Ching *et al*., 2014), white button turmeric with maceration and soxhlet methods using water solvent resulted in yield extract of 11% (Saputra, 2016), yellow turmeric extraction with soxhlet method using methanol resulted in yield extract of 5.6% (Kulkarni *et al.,* 2017), and according to (Wati *et al*., 2019), white turmeric extraction with soxhlet method using ethanol resulted in yield extract of 12.56%. Based on the results that were reported of the extractions of different kinds of plants and solutions, there is no activity test on the anticancer on the P-388 murine leukemia cells. Research needs to be done on the extraction to get curcuminoids with extraction time, different kinds of solvents and the solvent concentration, activity test on the P-388 murine leukemia cells, and pH testing of the yellow turmeric ethanol extract.

Research Method

Tools

The equipment used in the chemical laboratory consists of Erlenmeyer, measuring cups, drop pipettes, beakers, funnels, thermometers, and stirring rods. Other supporting tools are a set of soxhlet tools, rotary evaporator R-200 Buchi with a vacuum pump Vac V-500 Buchi, water bath B-490 Buchi, and UV-Visible Thermo Genesis 20 equipment.

Materials

The yellow turmeric (*Curcuma longa* L) is brought from Ciamis. Ethanol 70 and 96%, universal pH, the standard curcumin from PT Merck (Jakarta – Indonesia), and a set of anticancer activity materials P-388 murine leukemia cells are from CV Diva Pharmalab Supplier (PT Indogen Intertama. Jakarta – Indonesia).

Sample Preparation

The sample used in this research is yellow turmeric (*Curcuma longa* L) brought in from Ciamis, which is then determined in the School of Life Sciences and Technology – Bandung Institute of Technology. About 3 kgs of yellow turmeric are washed in water, cut, and dried in the oven at a temperature of 40 ^{o}C. The dried yellow turmeric is then pounded until it is fine with the size of 70/120 mesh.

Yellow Turmeric Ethanol Extraction (Soxhlet Method)

The extraction is conducted in several different conditions and repeated two times (n=2). The variables include raw materials with the solvent, the concentration of the solvent, and extraction time. The solvent used is solvent 70 and 96%, the comparison of the yellow turmeric powder and the solvent is 1:8, and the operation is conducted for 3, 6, 9, and 12 hours.

30 grams of yellow turmeric powder is put into a soxhlet and solidified. Put a piece of filter paper on top of the turmeric powder to avoid the sample from

being mixed with the solvent, and then the extraction is conducted at the temperature of 78˚C, the turmeric powder and solvent with the comparison of 1:8, the varied operation time of 3, 6, 9, and 12 hours. The extract resulting from this extraction is wet as it is still mixed with its solvent, so another separation process using a rotary evaporator is needed to get dried extract, which is then weighed to get the yield extract.

Analyzing the Result Percentage of Curcuminoid

Curcuminoid yield is used to know how much yellow turmeric extract is obtained using the equation: (Sahne *et al.,* 2016)

$$\% \text{ yield} = \frac{\text{Extraction Result}}{\text{Mass sample}} \text{ x } 100\% \text{ ……..(1)}$$

Curcumin and Curcuminoid Analysis with Spectrophotometer UV-Visible

Making Curcumin Standard Curve

A standard curve is made by making standard curcumin solution with concentrations of 0.5 ppm, 1 ppm, 1.5 ppm, and 2 ppm. Each concentration is analyzed with the spectrophotometer UV-Visible in the maximum wavelength so we get absorbance for every concentration. The wavelength with the biggest absorbance value is the maximum wavelength that will be used to measure the absorbance sample.

b. Determining Curcuminoid Rate with Spectrophotometer UV-Visible

Each 0.003 gram of dried extract from the extraction result is solved into ethanol to dilute it. The spectrophotometer cuvette is filled with the solution from the dilution process and put into the spectrophotometer. The absorbance scale is read in the wavelength of 390 nm (seen from the peak of the active spectrum in the curcumin standard analysis). The curcumin concentration is measured with the absorbance vs. concentration standard curcumin chart, using the equation

$$y = mx \text{ ……. (2)}$$

$$\text{Concentration} = f_{dilution}.x \text{ ……(3)}$$

y = concentration
x = absorbance
m = gradient
f = dilution factor

Activity Test on P-388 Murine Leukemia Cells

Anticancer activity test on P-388 murine leukemia cells is conducted on yellow turmeric extract with an operation time of 9 hours. P-388 cells are bred in RPMI 1640 media which is complemented with 5% of fetal bovine serum (FBS) and kanamycin (100 µg/mL). The cell (3 x 103 cell/well) is cultured in a microplate which contains 100 µL of growth media for each well and incubated at

the temperature of 37°C in an atmospheric humidity of 5% CO_2 for 2 x 24 hours. Add 10 µL more of the sample. Add 20 µL of MTT solution (5 mg/mL) on the third day into the cultured media. After 4 hours of incubation, add 100 µL of 10% solution of SDS 0,01 N HCl into the well. (Anwar and Fitrya, 2009)

pH Test of Color Substance

The extraction result product which is curcuminoids in ethanol solvent is pH tested using universal pH. That pH is then varied to pH 2, 4, 6 by adding H_2SO_4 solution and to pH 9, 12 by adding NaOH solution. Observe the colors coming off of the variations of those pH.

Results and Discussions

The yield of the Extraction Results

The raw materials that will be used in this research are firstly determined to decide the species of yellow turmeric. The result of the determination shows that the turmeric used is *Curcuma longa* L. Before the extraction process, a set of initial processes is conducted, which consists of peeling, cleaning, drying, and refining, to get yellow turmeric powder with the size of 70/120 mesh. About 30 gr of the refined yellow turmeric powder is extracted with the soxhlet method using varying concentrations of ethanol solvent used in the extraction process which are 70 and 96%, operation time of 3, 6, 9, 12 hours, the ratio of raw materials to solvent 1:8. The result percentage obtained from this research with different time variations is shown

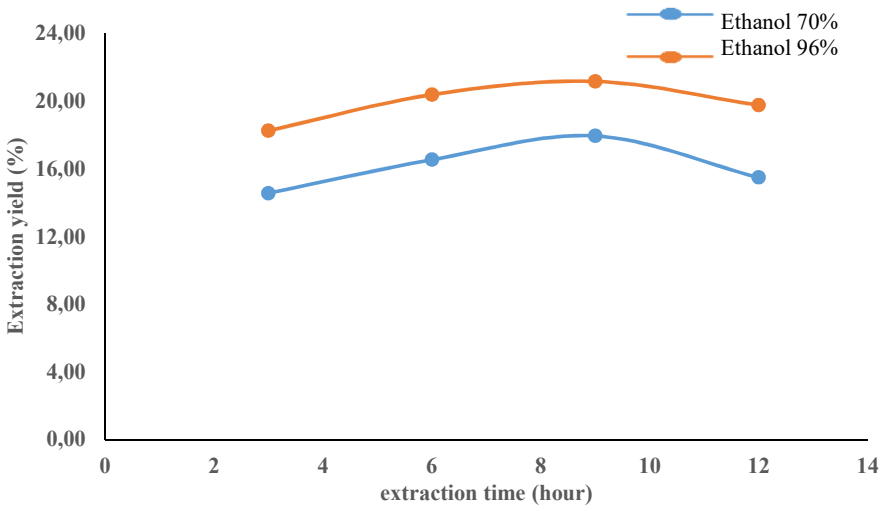

Figure 1. Chart Yield of Extraction Results to Extraction Time

Figure 1 shows the chart of the yield of the extraction results with the extraction time. The extraction time shows the length of the contact between the solution to be extracted and the solvent used so it affects the amount of the

extracted curcuminoid (Patel *et al*., 2019). The yielded result with the optimum time obtained at the operation time of 9 hours using ethanol solvent of 70 and 96% resulting in the biggest curcuminoid of 17.93 and 21.15%. This is shown by the changing of colors from brown to colorless when there is contact between the solvent and raw materials. This result means that the extraction process is in balance or reaches the solvent's maximum ability to extract the substances in the yellow turmeric. Several research results report that (Kulkarni *et al.,* 2017) the extraction of yellow turmeric with methanol solvent in the operation time of 7 hours results in the yield of 5.6%, (Verma, 2014) used methanol : water solvent with the operation time of 24 hours getting yield extract of 10.84%, (Oktavianingsih *et al.,* 2018) ethanol 70% with an extraction time of 3 and 4 hours resulting in yield 0.0871% and 0.0884%, while on ethanol 90% with an extraction time of 5 hours giving the highest yield that is 0.1764%. According to (Wati *et al*., 2019) for the extraction of white turmeric, the yield was obtained using ethanol 96% and ethanol 70% with an operation time of 17 hours, the highest values are 12.56% and 12.22%.

The connection between the extraction time and the amount of curcuminoid being extracted is in direct proportion. The longer the extraction time, the more curcuminoid is extracted so the extraction yield is high. This condition will continue until the curcuminoid concentration in the raw materials and the curcuminoid in the solvent are in balance (Patel *et al*., 2019; Singh *et al.*, 2017). figure 1 shows that in the time 9-12 hours there is a decrease in the curcuminoid yield. This might happen because the curcuminoids compound in yellow turmeric is unstable over light, temperature, pH change, and the long extraction time which cause the degradation of curcuminoid by forming ferulic acid and feruloyl methane (Nurhadi, 2012; Oktavianingsih *et al.,* 2018), so the curcuminoid obtained decreases in the time of 12 hours. The highest yield of curcuminoid is 21.15% in the operation time of 9 hours using ethanol solvent 96%.

Curcumin Concentration

The measurement of curcumin concentration is obtained from the spectrophotometry UV-Visible results, after the maximum wavelength is determined by comparing the obtaining of curcumin on the extract from soxhlet extraction with the standard curcumin, then make a curve between absorbance and concentration on the maximum wavelength of 390 nm. Figure 2 shows the chart on the relation between the absorbance and concentration with the variation of standard curcumin solution of 0.5-2 ppm taken from the equation chart with line y = 0.9567x. The line from the result of the equation that is obtained is then used to measure the curcumin concentration.

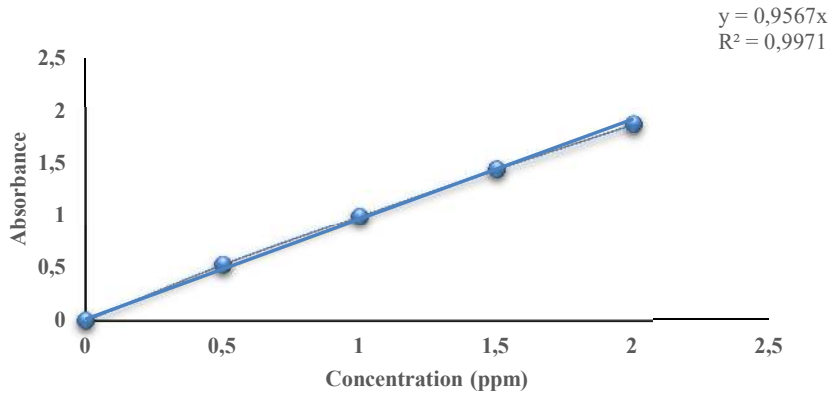

Figure 2. Chart The Relation Between Absorbance and Standard Curcumin Concentration

Figure 3 shows the chart of curcumin concentration with the extraction time. The biggest result of the curcumin concentration on yellow turmeric is 1121.10 and 1043.05 ppm on the optimum time with the operation time of 9 hours with ethanol solvent 96% ethanol 70%. Higher ethanol concentration can solve more curcumin. So the extraction using a solvent with an ethanol concentration of 96% gets higher curcumin compared to the ethanol concentration of 70%. The result matches the study of (Oktavianingsih *et al.*, 2018) the curcumin concentration of yellow turmeric with ethanol 90% and the longer the extraction shows the increase of the curcumin rate that is 2.47%, on ethanol 70% is a bit more, 0.344%, and (Ihsan *et al.,* 2018) discloses the result of ethanol concentration of 96%, 80%, and 70% respectfully is 17.64%, 16.99%, and 4.95%. According to (Wati *et al*., 2019) the result of white turmeric concentration with ethanol 96% is bigger with the value of 3264.60 ppm compared to the ethanol 70% with 2582.02 ppm. The different ethanol concentration tends to experience an increase along with how high the ethanol concentration is being used. This is because curcumin is a compound that can be dissolved quite enough in organic solvent and dissolved well in ethanol solvent (Kautsari *et al.,* 2020).

Ethanol concentration shows how much the ethanol content is in the solvent. The bigger the ethanol concentration, the more curcumin will be extracted and the more curcuminoid that will be obtained. This is because curcumin has a high solubility in ethanol compared to water (Wahyuningtyas *et al.*, 2017). According to (Patel *et al*., 2019) and (Popuri, 2013), the high solubility of curcumin in ethanol allows curcumin to be extracted well where ethanol solvent is a better solvent compared to other hydrocarbon solvents.

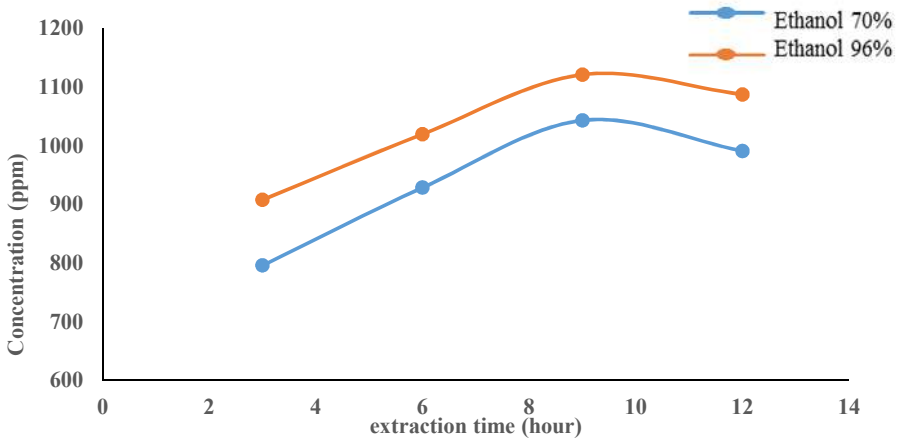

Figure 3. Chart Concentration on Extraction Time

Activity on P-388 Murine Leukemia Cells

Anticancer activity test on P-388 murine leukemia cells on yellow turmeric extract with operation time of 9 hours. That time is optimum and the curcuminoid compound obtained is the highest extract amount. The effectivity of a sample to ward off the free radicals uses a symbol of Inhibitory Concentration (IC_{50}) (Widyasanti *et al*., 2016). The result of the cytotoxic test is indicated by Inhibitory Concentration (IC_{50}) which shows the concentration of an extract or pure compound needed to inhibit the growth of cancer cells by as much as 50%. The strength of anticancer activity is indicated as follows (Anwar and Fitry, 2009)

1. IC50 5 µg/mL = very active
2. IC50 5-10 µg/mL = active
3. IC50 11-30 µg/mL = medium
4. IC50 >30 µg/mL = not active

Seeing how strong the resulting activity of yellow turmeric extract with an operation time of 9 hours, it has an activity on P-388 murine leukemia cells with the value of IC_{50} 6,15 µg/mL indicating that curcuminoid compound has the potential to be a leukemia anticancer that can inhibit the growth of cancer cells as much as 50%. (Kouhpeikar *et al*., 2019) reports that the anticancer activity of the curcumin compound in the curcuminoid obtained on the P-388 murine leukemia cells with the MTT method is thought to have a hydroxyl group role, and the methoxy group which is bound on the aromatic ring is an important factor in the cytotoxic activity. A molecule with bioactivities contains one or more functional groups in a certain position so it can precisely bind a function group on target macromolecules or receptors. According to (Rusanti *et al.,* 2017) the toxic characteristic of a plant can be caused by phenol compound with hydroxyl group like the curcumin compound in the curcuminoid that can inhibit the growth of leukemia cancer cells with the value of IC_{50} 19,21 µg/mL. (Musfiroh *et al*., 2020)

reveals the result of the activity test of yellow turmeric essential oil on P-388 leukemia cells that shows that 50% of P-388 leukemia cells can be inhibited by the yellow turmeric essential oil with the concentration of IC_{50} 15,5 µg/mL. (Eryanti *et al*., 2012) reveals that the test result of P-388 with the value of IC_{50} 17 µg/ml shows that curcumin derivative compounds (dibenzylhexahexanon) is a potential compound to inhibit the growth of leukemia anticancer.

The curcumin anticancer activity is associated with its ability to be a COX inhibitor or on the cell signalling track, whether it is through apoptosis or cell cycle arrest by affecting the tumor suppressor gene product or oncogenes. And also as antioxidant cell proliferation inhibitor, anti-inflammation, carcinogenesis inhibition, immunomodulatory, antiestrogen, and antiangiogenesis. (Mutiah, 2015). The mechanism of curcumin is through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) induction inhibition. The nuclear factor kappa B (NF-kB) is a form of protein in a cell cytoplasm which is bound in an inactive form with the function to regulate inflammation, immune responses, wound healing, death, and cell functions. *(*Supriano *et al.,* 2018)

pH Testing on the Sample Color Change

pH testing is done to analyse the stability of curcuminoid colors. Colors on curcuminoid are pH function, so the colors are different depending on the pH solution and can be used as a natural dye (Koswara, 2009). Testing the pH colors is done to the result of the extraction that gives the highest concentration and yield with the operation time of 9 hours using ethanol solvent 96%. Image 4 shows that the result of curcuminoids pH 6 has a bright yellow color. The acid extract added to H_2SO_4 reaches pH 2 and 4 giving off deeper yellow color that has higher intensity compared to a more neutral pH. Extract on alkaline pH added to NaOH until it reaches pH 9 resulting in a brownish color and pH 12 is a reddish color. Curcuminoid pH 8.5-10 experiences degradation forming vanillin, ferulic acid, and feruloyl methane (Sugih *et al.,* 2016). According to (Sundari, 2016), curcumin in acid is light yellow and alkali is brownish red. Yellow and red substances are natural coloring for food. Curcumin gives a clear and quick change of colors in less than 5 seconds making it possible as an indicator. (Dewi and Rusita, 2017) reports a study result that a stable pH which is 4, a stable pH value of a solution, shows that the distribution process of basic materials in the preparation spreads evenly. The recommended pH value for syrup is between 4-7.

Curcuminoid is a compound from the phenolic group which consists of curcumin, monodesmethocurcumin, and bidesmethocurcumin. A particular compound that gives yellow color is curcumin (1,7-bis (‘hydroxy-3 methoxyphenyl)-1,6 heptadien, 3,5-*dion* (Wahyuni 2016). Curcuminoid that is observed visually is pale yellow (Adawiyah *et al*. 2019), in accordance with the pH 4 result that can be seen in figure 4.

Figure 4. Results of Color Substance Test on pH 2, 4, 6, 9, and 12

Conclusion

From the analysis, we get that yellow turmeric extraction using ethanol solvent 96% with the operation time of 9 hours resulting in the highest yield that is 21.15% with the concentration of 1121.10 ppm. Curcuminoid compound with operation time of 9 hours has activity on P-388 murine leukemia cells with the value of IC_{50} 6,15µg/mL that has the potential to be leukemia anticancer that can inhibit the growth of cancer cells as much as 50%, and the color substance analysis on acidic pH (2, 4, 6) is yellow, on alkaline pH (9, 12) is brownish red as food natural coloring.

Acknowledgements

This research is supported by the Institute of Research and Community Services (or Lembaga Penelitan dan Pengabdian Masyarakat which is shortened as LPPM), the Institut Teknologi Nasional, Bandung Nomor: 312/B.05/LPPM-Itenas/V/2021, through the Itenas Grant to Middle Lecturers.

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