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Acetylcholinesterase Enzyme Inhibitor and Antioxidant Activities from A Mixture Extracts of Black Tea, Red Betel, Cinnamon and Curcuma

Aziz Syamsul Huda[1], Akhmad Endang Zainal Hasan[1*], Mega Safithri[1]

[1]*Department of Biochemistry, IPB University, Bogor, 16680, Indonesia*

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Corresponding author : Akhmad Endang Zainal Hasan, Departemen Biokimia FMIPA IPB;
e-mail: zainalhasan@apps.ipb.ac.id

ABSTRACT

Alzheimer's Disease (AD) is one of the consequences of impaired functioning of acetylcholine which can be hydrolyzed by the enzyme acetylcholinesterase. Alzheimer's treatment is carried out using two approaches, namely compounds (acetylcholinesterase inhibitors) and noncholinergics (antioxidants). There are commercial drugs that can slow the progression of Alzheimer's, but their use can cause excessive side effects. The use of herbal plants as a cure for Alzheimer's disease has been proven to be safer and does not cause excessive side effects. Herbal plants that can be used and developed are black tea, red betel, curcuma, and cinnamon. This study determined that extracts of black tea, red betel leaf, curcuma, and cinnamon's formula have the best antioxidant activity and level of inhibition of the acetylcholinesterase enzyme. F1 (cinnamon), F5 (cinnamon, black tea, red betel, and curcuma), F6 (cinnamon and black tea), and F11 (cinnamon, black tea, and curcuma) were the best formulations in each type of sample based on inhibitory AChE enzymes and antioxidants activity. F1 (cinnamon) is the most effective extract out of all formulations analyzed in this study.

Keywords: *Acetylcholinesterase; Alzheimer; Black Tea; Cinnamon; Red Betel; Curcuma*

1. INTRODUCTION

Alzheimer's Disease (AD) is a result of impaired acetylcholine function. This nerve excitation conductor (neurotransmitter) can be hydrolysed by the acetylcholinesterase enzyme. Acetylcholinesterase (AchE) is an enzyme that functions as a catalyst in the breakdown of acetylcholine (AChE). In many people with dementia, the activity of the acetylcholinesterase enzyme is greater, so compounds that can inhibit AChE activity can be described as a potential anti-dementia drug (Safwan *et al*. 2014). AChE inhibitors are used in the treatment of dementia for the cholinergic approach. Meanwhile, a non-cholinergic approach can be done through the use of antioxidants (Surya 2013). Antioxidants are compounds that react by eliminating, taking up, supressing the formation and suppressing the activity of radical species (oxidants) (Nirwana & Mutakin 2019).

Red betel (*Piper Crocatum*) and cinnamon (*Cinnamon Burmaii*) are plants have the potential to be developed as an alternative to

Alzheimer's treatment. The active compound in red betel leaf can inhibit the AChE enzyme in silico (Syifa 2021). Previous reseach has stated that red betel-based functional drinks have antioxidant activity (Safithri *et al*. 2020). In addition, there are flavonoid compounds (flemiphilippinin B or glabrescione B) and tannins (catechins or gallocathecins) in red betel extract which are considered to contribute to antioxidant activity (Safithri *et a*l. 2016). Cinnamon has potential as an alternative therapy for Alzheimer's related to inhibition mechanism of acetylcholinesterase enzyme. Based on the *In Silico* method, epicatechin, medioresinol, and gamma eudesmol compounds in cinnamon extract can inhibit the AChE enzyme (Syarafina *et al*. 2022).

Black tea (*Camellia sinensis*) and curcuma (*Curcuma Xanthorriza*) are plants having antioxidant properties. The antioxidant activity of black tea and curcuma have been proven *in vitro* and *in vivo*. Several in vitro methods have been carried out to test the antioxidant activity of black tea extract, including DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) (Hajiaghaalipour *et al*. 2016). The antioxidant activity of curcuma extract in vitro has been proven by the DPPH method (Widyastuti *et al*. 2020). Several parameters observed in the *in vivo* antioxidant activity test of aqueous black tea and curcuma extracts were SOD (Superoxide Dismutase) activity, GSH-Px (Gluthatione Peroxidase) activity, and MDA (Malondialdehyde) levels (Lukitaningsih *et al*. 2020; Sun *et al*. 2012).

The combination of the four plants is expected to be able to form a positive and synergistic interaction between antioxidant activity and AChE enzyme inhibitory activity so as to increase its potential as an alternative ingredient for a combined cholinergic and noncholinergic Alzheimer's treatments. In addition, the solvent that is used in material extraction is water. The use of water as a solvent is due to its relatively cheap price, easy to obtain, and relatively safe to use *(Novita et al.* 2016). Water solvents are relatively safer to use for the extraction of consumptive materials (Agustina 2017). This study is aimed to determine the optimal formulation for a mixture of black tea, red betel leaf, cinnamon and curcuma extracts which have the best antioxidant activity and inhibition of the acetylcholinesterase enzyme in vitro.

2. METHODOLOGY

The tools used were glassware, oven, blender, sieve, analytical balance, thermometer, water bath, desiccator, wood clips, test tube, vortex, filter paper, vacuum, funnel, magnetic stirrer, UV-VIS spectrophotometer, Mohr pipette, micro pipette, dropper, tip. The tool used for statistical data processing is a computer with Minitab software installed.

The materials used were distilled water, black tea simplicial, red betel leaf, curcuma and cinnamon, Folin-Ciocalteu reagent 10%, Na_2CO_3 10%, distilled water, gallic acid, methanol, trolox, ascorbic acid, NaOH 1M, DPPH 0.4 mM, and an AChE enzyme assay kit.

Extract Preparation

Materials simplicia are extracted by using an infusion method. The solvent used was distilled water with a ratio of 1:40 (black tea, curcuma and cinnamon) and 1:20 (red betel). Extraction was carried out three times. The obtained filtrate is stored for further formulation and analysis.

Determination of Mixture Formula

The mixture formula was created by using the help of Design Expert 13 software with the model used was mixed optimization. The determination of each ingredient in the formula was carried out using the Simple Lattice Design (SLD) method. Simple Lattice Design is an optimization method used to determine the optimum formula for a mixture of ingredients with the proportion of the total amount of

different ingredients being 1 (100%). The materials or factors used in the optimization are at least two different materials. Factors in the mixture design will determine the design space or test area (Hidayat *et al*. 2020). The models produced 25 formulas which are divided into 4 types of formulas, namely 4 single ingredient formulas, 12 two-ingredient mixture formulas, 4 three-ingredient mixture formulas and 5 four-ingredient mixture formulas (Table 1).

Acetylcholinesterase (AChE) Enzyme Inhibition Test (Abcam: ab138871)

Sample measurements were carried out with three types of solutions, namely sample solutions, blank solutions, and negative standards. Samples were incubated for 30 minutes in the dark, then measured spectrophotometrically at a wavelength of 408 nm. The composition of each solution is shown in table 2. Standard curve was made by mixing dd H2O 50 µL, Ache Seri 50 µL and Acth mix 50 µL. AChE was made in several concentrations, namely, 300, 100, 30, 10, 3, 1 and 0 mU/mL. then it was incubated for 30 minutes in the dark, then measured spectrophotometrically at a wavelength of 408 nm.

Antioxidant Activity Testing using DPPH Method (Cottica *et al*. 2011)

The antioxidant activity of the sample was tested by measuring the inhibition of DPPH free radical oxidation. Each sample of 100 µL was reacted with 100 µL DPPH 125 µM. Then incubated for 30 minutes. After that, the absorbance was measured at a wavelength of 517 nm with nano-spektrofotometri (SPECTROstarNano BMG LABTECH). The standard used to create the calibration curve is trolox. Trolox as standard was used a range of concentrations (0, 10, 20, 40, 50, 80, 90, 100 µM).

Determination of Total Phenolic (Khumaida *et al*. 2019)

The total phenol content was determined using a microplate reader based on the Folin-Ciocalteu method. A total of 20 µL of the sample was put into a 96 well microplate. Then 120 µL of folin ciocalteu (10%) and 80 µL of Na_2CO_3 (10%) solution were added. The samples were incubated for 30 minutes in the dark at room temperature, then the absorbance of all samples was measured using a microplate reader at 750 nm (Khumaida et al., 2019). The standard used to create the calibration curve was the gallic acid standard. Gallic acid was used as standard at various concentrations (0, 50, 75, 100, 150, 200, 225 mg/L). The total phenolic content was expressed as mg gallic acid equivalent per mL of sample (mg GAE mL^{-1}). All samples were analyzed in three replicates.

Data Analysis

Data analysis was carried out using statistical methods to see the effect of samples and controls on the power of AChE inhibition activity. Statistical analysis was performed with Minitab software. The method used was a one-factorial completely randomized design (CRD), namely One-Way ANOVA. Then proceed with the Tukey Test if the results show a significant difference at the 95% level of significance. The $p<0.05$ value indicates a significant difference. The real effect of the treatment is expressed in different letter codes.

Table 1 Testing Formulations

Sample Number	Black Tea (%)	Red Betel (%)	Curcuma (%)	Cinnamon (%)
1	0,00	0,00	0,00	100,00
2	0,00	33,33	66,67	0,00
3	33,33	33,33	33,33	0,00
4	66,67	0,00	33,33	0,00
5	12,50	12,50	12,50	62,50
6	33,33	0,00	0,00	66,67
7	12,50	62,50	12,50	12,50
8	0,00	0,00	66,67	33,33
9	62,50	12,50	12,50	12,50
10	12,50	12,50	62,50	12,50
11	33,33	0,00	33,33	33,33
12	0,00	0,00	33,33	66,67
13	0,00	33,33	0,00	66,67
14	33,33	66,67	0,00	0,00
15	33,33	0,00	66,67	0,00
16	33,33	33,33	0,00	33,33
17	100,00	0,00	0,00	0,00
18	0,00	33,33	33,33	33,33
19	0,00	66,67	33,33	0,00
20	66,67	33,33	0,00	0,00
21	0,00	66,67	0,00	33,33
22	25,00	25,00	25,00	25,00
23	0,00	100,00	0,00	0,00
24	66,67	0,00	0,00	33,33
25	0,00	0,00	100,00	0,00

Table 2 Test Solution Composition

Type	dd H_2O (µL)	AChE 100 U/mL (µL)	ACTh mix (µL)	Extract Sample (µL)	Buffer (µL)
Sample	-	50	50	50	-
Control Negative	50	50	50	-	-
Blank	50	-	50	-	50

3. RESULT

AchE Inhibitory Activity

The results of the analysis showed that all samples of the tested formulas appeared to have inhibitory abilities ranging from 7.586% to 60.621% (Figure 1). The highest value was showed by by the F1 sample which contained a single ingredient of cinnamon. Cinnamon had a major effect on the AChE inhibitory activity of the formula. This can be seen from the inhibition values of samples F6 (cinnamon and black tea), F12 (cinnamon and curcuma), and F13 (cinnamon and red betel) which have higher inhibition values than the single ingredients (black tea, curcuma, and red betel).

Antioxidant Activities (DPPH)

The results of the antioxidant activity analysis showed that all samples had different antioxidant activities in each formula. A significant difference was shown by each sample after being tested by ANOVA test ($p<0.05$) and Tukey's follow-up test. The antioxidant activity of the sample ranged from 0.280 to 1.007 µmol TE/mL of the sample (Figure 2). The highest value was obtained by formula 11 which contains black tea, curcuma, and cinnamon. This value is higher than that of each individual ingredient, namely black tea (F17), red betel (F23), and cinnamon (F1). Formula 22 which is a combination of four ingredients has an antioxidant activity value of 0.947 µmol TE/mL. This value is higher than the value of the antioxidant activity of each ingredient. This shows that the combination of ingredients can increase its antioxidant activity.

Total Phenolic Level

Total phenolic analysis result showed that all samples contained phenolic level of various concentrations. A significant difference displayed by each sample after being tested by ANOVA test ($p<0.05$) and Tukey's follow up test. The phenolic content of the samples ranged from 0.066 to 0.677 mg GAE/mL sample (Figure 3). The highest value was obtained by F24 (black tea and cinnamon), while the lowest value was obtained by F25 (curcuma). Based on the result of the study, black tea was the ingredient that contributed the most to the increase in phenolic levels in the formula. This can be seen from the phenolic content of F24 which is higher than the total phenolic content of the individual extract samples, namely F1 (cinnamon) and F17 (black tea). In addition, there was an increase in phenolic levels in F4 and F20 compared to the individual extracts (F23 and F25). The increase was caused by an increase in the volume of black tea extract which contributed to the addition of the amount of phenolic in the combined extract.

Sample Optimization

Optimization was carried out by evaluating the formula based on the inhibitory activity of the AChE enzyme and antioxidant activity. The inhibitory activity of the AChE enzyme was used as the main parameter in determining the optimum formula. Formula 1 (cinnamon) is the best single ingredient formula based on AChE enzyme inhibition (60.621%) and antioxidant activity (0.902 mol TE/mL) (Figure 4). This value is higher than the inhibition value of the AChE enzyme in other formulas with single ingredients, namely F17 (black tea), F23 (red betel), and F25 (curcuma).

Formula 6 (cinnamon and black tea) is a formula with the best two mixtures based on AChE enzyme inhibition (54.207%) and antioxidant activity (0.918 µmol TE/mL) (Figure 4). Formula 11 (cinnamon, black tea, and curcuma) is a formula with the best three ingredients based on AChE enzyme inhibition (42.517%) and antioxidant activity (1.007 µmol TE/mL) (Figure 4). Formula 5 (cinnamon, black tea, red betel, and curcuma) is a formula

with the best four ingredients based on AChE enzyme inhibition (44.241%) and antioxidant activity (0.849 µmol TE/mL) (Figure 4).

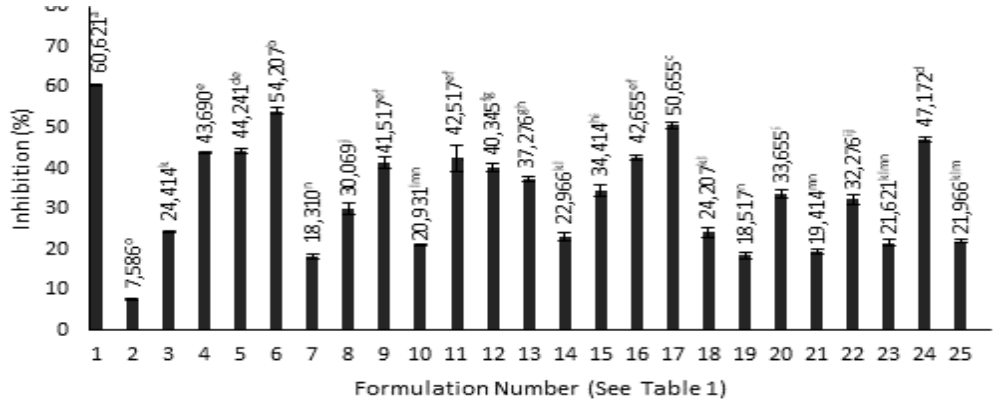

Figure 1 AchE Inhibition

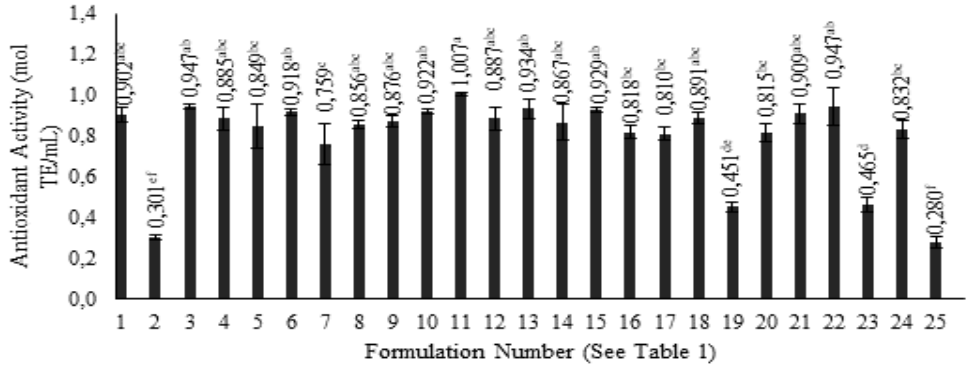

Figure 2 Antioxidant Activities

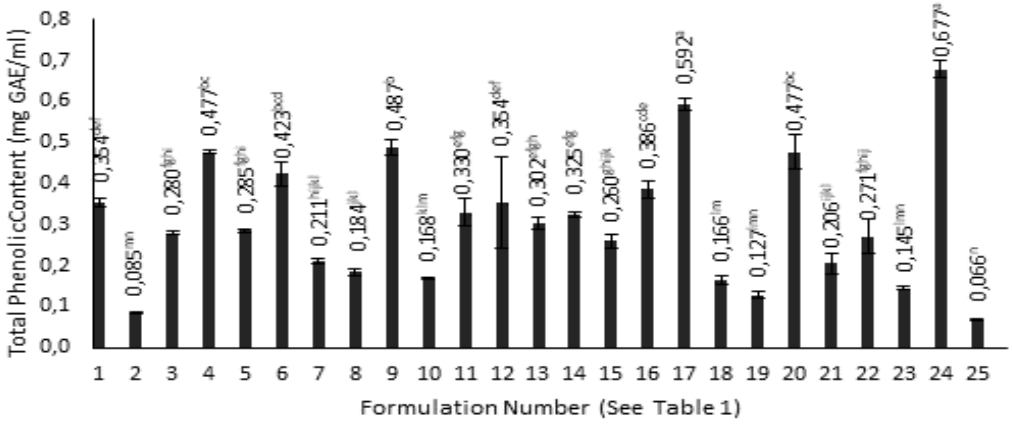

Figure 3 Phenolic Level

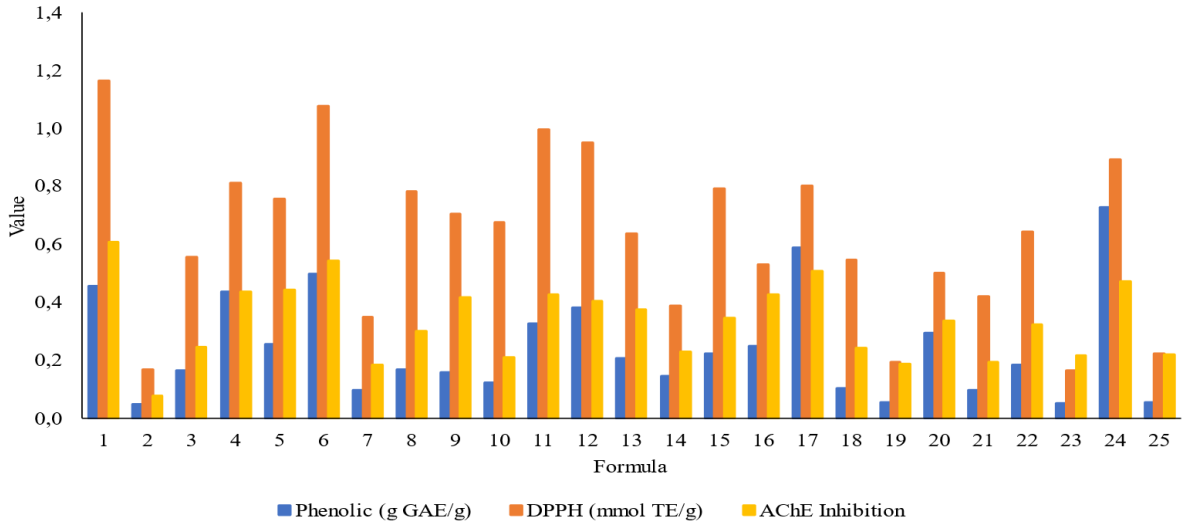

Figure 4 AChE Inhibitory Activities, Antioxidant Activities dan Phenolic Levels

4. DISCUSSION

AChE Inhibitory Activity

Tests on the formulas of the four ingredients showed the best results at F5 with an inhibition value of 44.241%, although for the single compounds cinnamon and black tea showed better results than red betel and curcuma. This effect is possible because of the interaction between the active compounds in the extract. This is possibly because there is no synergistic effect of the active compounds contained in the sample mixture so that the inhibition value is not better, because the extract used in this study is an impure crude extract and is suspected to contain other unidentified compounds that interfere with the work of the compound. In addition, it is possible that there is an antagonist compound in the extract of one of the ingredients that inhibits the action of the compound in the formula when the concentration is higher (Putri *et al.* 2017).

Changes in the volume ratio of cinnamon in the formula were seen to increase the inhibitory activity of the formula. This can be seen from the inhibition values of the other four ingredient formulas (F7, F9, F10, and F22) that were lower than F5 which was dominated by cinnamon. This condition may be due to the presence of a compound in one of the samples that creates antagonism. Antagonism is a state of interfering with or inhibiting each other's work or chemicals that interfere with other chemicals when given together or combined (Darwis *et al*. 2012) .

Antioxidant Activity

Antioxidant activity can be influenced by phytochemical compounds contained in the formula. According to Pulung & Yogaswara (2016), polyphenol content can act as DPPH radical acceptors. Each ingredient has secondary metabolites that have the potential to interact with each other when combined. Compounds other than the active substance as the main component may potentially affect the response of antioxidant activity. This difference in antioxidant activity is thought to be due to the distribution of the number and type of secondary metabolite compounds with antioxidant properties contained in the test sample (Huliselan *et al.* 2015).

The combination of phytochemical compounds can increase antioxidant activity through the regeneration mechanism of inactive antioxidant compounds. When antioxidant compounds have donated protons to stabilize radical compounds, these compounds are no longer active as antioxidants. Through a combination of phytochemical compounds, the

regeneration mechanism of antioxidant compounds can occur, even though the antioxidant activity of one of the ingredients is very low (Aftab & Vieira 2010).

Total Phenolic Level

Previous research stated that the phenolic content of black tea was 367.44 mg GAE/g, cinnamon was 121 mg GAE/g, red betel was 142.56 mg GAE/g, and curcuma was 3.87 mg GAE/g (Almulki 2019; Ereifej *et al.* 2016; Prayitno *et al.* 2018; Suryani *et al.* 2022). The difference in phenolic content with previous studies can be caused by the type of solvent. The choice of solvent greatly affects the content in the plant. Solvents such as methanol and ethanol have been widely used for the extraction of phenolic from plants compared to using water. Total flavonoid, phenolic and antioxidant activity of limnophila aromatica extract using pure acetone and pure ethanol as solvents were higher than those using methanol and water (Do *et al*. 2014). Even so, the use of water is very effective because it can be consumed directly by the community.

Sample Optimization

The high inhibitory activity of the AChE enzyme in F1 could be caused by the phytochemical content in the cinnamon extract which can interact with the AChE enzyme and have an inhibitory effect. The epicatechin, medioresinol, and γ-eudesmol compounds in cinnamon extract can inhibit the AChE enzyme in Silico (Syarafina *et al.* 2022). The presence of black tea in F6 had a positive effect on the antioxidant activity of the formula so that the antioxidant activity value of F6 was higher than that of F1. This could be due to the presence of theaflavin and thearubigin compounds which play an important role in the antioxidant activity of black tea (Bhuyan *et al.* 2013). In addition, theaflavins and thearubigin have been shown to inhibit the AChE enzyme in vivo (Ray & De 2012).

The addition of curcuma in the formula had a positive effect on antioxidant activity. Curcuma has been shown to have antioxidant activity. Curcuma extract has an antioxidant activity of 5.034 µmol TE/g (Suryani *et al.* 2022). The addition of red betel extract had a positive effect on the inhibitory activity of the AChE enzyme where the inhibition value of the F5 AChE enzyme was higher than that of F11 without red betel. This can be caused by the phytochemical content in red betel that is able to inhibit the AChE enzyme. The compounds copaene, auron, and 1,1'-(6,6'-dihydroxy-5,5'-dimethoxy-3,3'-biphenyldiyl) di (1-propanone) in red betel extract have the ability to inhibit the AChE enzyme in silico. In addition, these three compounds can interact with CAS or PAS residues which play an important role in inhibiting the AChE enzyme (Syifa 2021).

All formulas have varying phenolic content, antioxidant activity and AChE enzyme inhibitory activity. F1 (cinnamon), F5 (cinnamon, black tea, red betel, and curcuma), F6 (cinnamon and black tea), and F11 (cinnamon, black tea, and curcuma) were the best formulas in each type of formula based on inhibitory activity AChE enzymes and antioxidants. F1 (cinnamon) is the best formula of all formulas analyzed in this study.

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CONFLICT OF INTEREST

The authors declared that they have no competing interests.

REFERENCES

Aftab, N., & Vieira, A. (2010). Antioxidant Activities of Curcumin and Combinations of this Curcuminoid with other Phytochemicals. *Phytotherapy Research*, *24*(4), 500–502. https://doi.org/10.1002/ptr

Agustina, E. (2017). Uji Aktivitas Senyawa Antioksidan Dari Ekstrak Daun Tiin (Ficus Carica Linn) Dengan Pelarut Air, Metanol Dan Campuran Metanol-Air. *Klorofil : Jurnal Ilmu Biologi Dan Terapan*, *1*(1), 38–47. http://jurnal.uinsu.ac.id/index.php/klorofil/article/view/1240

Almulki, W. (2019). *Optimasi suhu dan campuran ekstrak air teh putih, teh hijau dan teh hitam terhadap total fenolik dan total flavonoid*. IPB University.

Bhuyan, L. P., Sabhapondit, S., Baruah, B. D., Bordoloi, C., Gogoi, R., & Bhattacharyya, P. (2013). Polyphenolic compounds and antioxidant activity of CTC black tea of North-East India. *Food Chemistry*, *141*(4), 3744–3751. https://doi.org/10.1016/j.foodchem.2013.06.086

Cottica, S. M., Sawaya, A. C. H. F., Eberlin, M. N., Franco, S. L., Zeoula, L. M., & Visentainer, J. V. (2011). Antioxidant activity and composition of propolis obtained by different methods of extraction. *Journal of the Brazilian Chemical Society*, *22*(5), 929–935. https://doi.org/10.1590/S0103-50532011000500016

Darwis, W., Hafiedzani, M., & Astuti, R. R. S. (2012). Efektivitas ekstrak akar dan daun pecut kuda Stachytarpetha jamaicensis Vahl dalam menghambat pertumbuhan jamur Candida albicans penyebab kandidiasis vaginalis. *Konservasi Hayati*, *8*(2), 1–6.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of Food and Drug Analysis*, *22*(3), 296–302. https://doi.org/10.1016/j.jfda.2013.11.001

Ereifej, K. I., Feng, H., Rababah, T. M., Tashtoush, S. H., Al-U'datt, M. H., Gammoh, S., & Al-Rabadi, G. J. (2016). Effect of Extractant and Temperature on Phenolic Compounds and Antioxidant Activity of Selected Spices. *Food and Nutrition Sciences*, *07*(05), 362–370. https://doi.org/10.4236/fns.2016.75038

Hajiaghaalipour, F., Sanusi, J., & Kanthimathi, M. S. (2016). Temperature and time of steeping affect the antioxidant properties of white, green, and black tea infusions. *Journal of Food Science*, *81*(1), 246–254. https://doi.org/10.1111/1750-3841.13149

Huliselan, Y. M., Runtuwene, M. R. J., & Wewengkang, D. S. (2015). Aktivitas Antioksidan Ekstrak Etanol, Etil Asetat, Dan n-Heksan Dari Daun Sesewanua (Clerodendron squamatum Vahl.). *Pharmacon*, *4*(3), 155–163.

Khumaida, N., Syukur, M., Bintang, M., & Nurcholis, W. (2019). Phenolic and flavonoid content in ethanol extract and agro-morphological diversity of Curcuma aeruginosa accessions growing in west java, Indonesia. *Biodiversitas*, *20*(3), 656–663. https://doi.org/10.13057/biodiv/d200306

Lukitaningsih, E., Rohman, A., Rafi, M., Nurrulhidayah, A. F., & Windarsih, A. (2020). In vivo antioxidant activities of Curcuma longa and Curcuma xanthorrhiza: A review. *Food Research*, *4*(1), 13–19. https://doi.org/10.26656/fr.2017.4(1).172

Nirwana, A. C., & Mutakin. (2019). Aktivitas antioksidan dari suku Rutaceae. *Farmaka*, *17*(1), 66–76.

Novita, M., Sulaiman, M. I., & Yura, S. (2016). Pengaruh Jenis Pelarut terhadap Aktivitas Antioksidan dan Kandungan Fenol Beberapa Jenis Bayam dan Sayuran Lain (Effect of Solvent Extraction on Antioxidant Activity and Phenolic Content of Variety of Amaranth and Other Vegetables). *Jurnal Ilmiah Mahasiswa Pertanian Unsyiah*, *1*(1), 935–940.

Prayitno, S. A., Kusnadi, J., & Murtini, E. S. (2018). Desember 2018, hal. 26-34 Karakteristik (Total Flavonoid, Total

Fenol. *Aktivitas Antioksidan) Ekstrak Serbuk Daun Sirih Merah*, *1*(2), 26–34.

Pulung, M. L., & Yogaswara, R. (2016). Potensi Antioksidan Dan Antibakteri Virgin Coconut Oil Dari Tanaman Kelapa Asal Papua. *Chemistry Progress*, *9*(2), 63–69.

Putri, R., Mursiti, S., & Sumarni, W. (2017). Aktivitas Antibakteri Kombinasi Temu Putih dan Temulawak terhadap Streptococcus Mutans. *Jurnal Mipa*, *40*(1), 43–47.

Ray, S., & De, B. (2012). Acetylcholinesterase inhibitory properties of black tea and its polyphenolic components. *International Journal of Pharmacy and Pharmaceutical Sciences*, *4*(3), 334–337. https://doi.org/10.13140/RG.2.2.33531.77601

Safithri, M., Indarianib, S., & Yuliani, R. (2020). Effect of Microencapsulation Techniques on Physical and Chemical Characteristics of Functional Beverage Based on Red Betel Leaf Extract (Piper crocatum). *Jurnal Kimia Sains Dan Aplikasi*, *23*(8), 276–282. http://ejournal.undip.ac.id/index.php/ksa

Safithri, M., Kurniawati, A., & Syaefudin. (2016). Formula of Piper crocatum, Cinnamomum burmanii, and Zingiber officinale extracts as a functional beverage for diabetics. *International Food*, *23*(3), 1123–1130.

Safwan, S., Yuliani, S., & Pramono, S. (2014). Uji aktivitas minyak atsiri rimpang kunyit (Curcuma longa Linn) pada tikus sprague dawley model demensia (kajian penghambat aktivitas asetilkolinesterase). *Kartika Jurnal Ilmiah Farmasi*, *2*(2), 20–26. https://doi.org/10.26874/kjif.v2i2.29

Sun, S., Pan, S., Ling, C., Miao, A., Pang, S., Lai, Z., Chen, D., & Zhao, C. (2012). Free radical scavenging abilities in vitro and antioxidant activities in vivo of black tea and its main polyphenols. *Journal of Medicinal Plants Research*, *6*(1), 114–121. https://doi.org/10.5897/jmpr11.1308

Surya, K. N. (2013). *Potensi Ekstrak Bakau (Rhizophora spp.) Sebagai Inhibitor Asetilkolinesterase dan Antioksidan*. IPB University.

Suryani, Marlin, Anshory, A. C., Artika, I. M., Ambarsari, L., & Nurcholis, W. (2022). Variability total phenolic content and antioxidant activity of Curcuma zanthorrhiza and C . aeruginosa cultivated in three different locations in West Java , Indonesia. *Biodiversitas*, *23*(4), 1998–2003. https://doi.org/10.13057/biodiv/d230434

Syarafina, Z. Y. I., Safithri, M., Bintang, M., & Kurniasih, R. (2022). In silico screening of cinnamon (Cinnamomum burmannii) bioactive compounds as acetylcholinesterase inhibitors. *Jurnal Kimia Sains Dan Aplikasi*, *23*(3), 97–107. https://scholar.archive.org/work/forwjldoafbenilgbyc4sxum2m/access/wayback/http://ejournal.undip.ac.id:80/index.php/ksa/article/viewFile/15913/11854

Syifa, K. (2021). *Analisis potensi senyawa aktif daun sirih merah (Piper crocatum) sebagai inhibitor enzim asetilkolinesterase secara in silico*. IPB University.

Widyastuti, I., Luthfah, H. Z., Hartono, Y. I., Islamadina, R., Can, A. T., & Rohman, A. (2020). Aktivitas Antioksidan Temulawak (Curcuma xanthorrhiza Roxb.) dan Profil Pengelompokannya dengan Kemometrik. *Indonesian Journal of Chemometrics and Pharmaceutical Analysis*, *1*(1), 29–42. https://doi.org/10.22146/ijcpa.507