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The Effect of $Zncl_2$ Concentrations and Heating Methods on the Chlorophyll, Phenolic, Andrographolide Content and Antioxidant Activity of Sambiloto (*Andrographis Paniculata*) Simplicia Powder

CHATARINA LILIS SURYANI[1], FX SUWARTA[2] and ICHLASIA AINUL FITRI[1]*

[1]Department of Food Technology, Faculty of Agroindustry, Universitas Mercu Buana Yogyakarta, Yogyakarta, Indonesia.
[2]Department of Animal Husbandry, Faculty of Agroindustry, Universitas Mercu Buana Yogyakarta, Yogyakarta, Indonesia.

Abstract

The drying process in Sambiloto simplicia production causes the degradation of chlorophyll as the major compound. However, the stability of chlorophyll can be enhanced by forming a metallochlorophyll complex with zinc (Zn) metal. This research aims to produce Zn-rich Sambiloto simplicia powder to produce high stability of chlorophyll. Sambiloto simplicia powder was prepared through sorting, grinding, sieving, and mixing with $ZnCl_2$ solution at concentrations of 0, 200, 300, 400, and 500 ppm, followed by heating (using either oven or an autoclave) and drying. The analysis included determination of total chlorophyll, phenolic, flavonoid, zinc, and andrographolide content. DPPH and FTC assays were employed to evaluate antioxidant activity. The results showed that increasing $ZnCl_2$ concentration up to 300 ppm enhances chlorophyll, phenolic, flavonoid, zinc, and andrographolide content. Higher concentrations of $ZnCl_2$ in the oven resulted in decreased lipid peroxidation inhibition (LPI) of Sambiloto simplicia powder, whereas using an autoclave led to increased LPI. Furthermore, higher $ZnCl_2$ concentrations, up to 400 ppm, corresponded to increased DPPH radical scavenging activity. Lipid peroxidation inhibition activity correlated with andrographolide, zinc, and total phenolic contents. Conversely, DPPH radical scavenging activity strongly correlated with total phenolic, total flavonoid, Zn, chlorophyll, andrographolide content.

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CONTACT Ichlasia Ainul Fitri ichlasia@mercubuana-yogya.ac.id Department of Food Technology, Faculty of Agroindustry, Universitas Mercu Buana Yogyakarta, Yogyakarta, Indonesia.

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Introduction

Changes in people diets, which contain more fat and calories especially during industrial growth may increase the risk of free radical exposure in the body. It is caused by the body's lack of antioxidants, so additional antioxidant intake from external sources is needed. Many antioxidants are developed from herbal ingredients because they are safer than synthetic ones. One herbal ingredient that has antioxidant activity is sambiloto leaf (*Andrographide paniculata*).[1] In Indonesian society, sambiloto herbs are used for brewing dry ingredients. However, the drying methods can cause a decline in the amount and activity of functional components, for example, phenolics,[2] flavonoids,[3] chlorophyll,[4] and andrographolide compounds.[5] One process known to reduce chlorophyll degradation is blanching with a $ZnCl_2$ solution.[6] However, the research results of Usman *et al*.[7] showed that the blanching process decreased total phenolic and chlorophyll content because much of it was dissolved in the blanching media. This is because the shape of the sambiloto leaf is thin and small. Therefore, this research carried out the metallochlorophyll formation process on simplicia sambiloto powder by mixing the $ZnCl_2$ solution with the material and continuing with the heating process.

There are several types of heating equipment, including ovens and autoclaves which are normally used to produce dry simplicia sambiloto. The temperature and heating time can be controlled in the oven heating process. The heat conduction medium in the oven is dry air and conduction from the container to the material. In autoclave heating, heat is delivered through the saturated steam and factors that control temperature, time, and water vapor pressure. The different heating principles will affect the level of degradation of functional components such as phenolics and their antioxidant activity.[8] Therefore, research has been carried out on the process of making sambiloto simplicia powder by forming a Zn-chlorophyll complex at various reagent concentrations and using two heating methods, namely the oven method and the autoclave method on the dried sambiloto leaf, to obtain sambiloto simplicia powder, which has high antioxidant activity. This research will be important especially to evaluate the influence of $ZnCl_2$ concentration and heating methods on the chemical properties and antioxidant activity of Simplicia sambiloto powder and to produce simplicia sambiloto powder rich in Zn, which has high antioxidant activity.

Materials and Methods

Materials

The material used in this research was dried simplicia from the Tawangmangu Medicinal Plant Research Institute. The chemical for forming metallochlorophyll complex is $ZnCl_2$ (Sigma, Aldrich). Chemicals for testing phenolic, flavonoid, and andrographolide content and antioxidant activity are gallic acid and quercetin standard, Folin-Ciocalteu reagent, linoleic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and butylated hydroxytoluene (BHT) from Merck with standard specifications for analysis (pro analysis).

Methods

Sample Preparation

The simplicia sambiloto was purchased from Tawangmanggu Medicinal Plant Research Institute. Sambiloto simplicia powder is made through sorting stages to remove the tough leaf stalks, grinding with a dry blender for 1 minute at three times, sieving (60 mesh), and crushing materials that do not pass through the sieve again. Intermittent grinding is carried out to avoid heat accumulation, which can cause degradation of the functional components.

Metallochlorophyll Complex Formation

The metallochlorophyll complex is formed by mixing simplicia powder with the reagent, continued by stirring, heating, and drying in a cabinet drier9. The reagent used is ZnCl2 with concentrations 0, 200, 300, 400, and 500 ppm. The ratio between ingredients and reagents is 50:15 (w/v). Mixing is done with a spray and stirring to ensure homogeneity. The mixture was then heated using two methods. These are heated with an oven (Memert) and an autoclave (All American on electric pressure, model 1925X) for 10 minutes at 110 °C. The sambiloto simplisia powder was dried again using a cabinet dryer at 50 °C for 3 hours and stored in plastic wrapped in aluminum foil.

Total chlorophyll

Total chlorophyll content (TCC) was measured using the Rababah *et al*., (2015).[3] A 5 g of sambiloto simplicia powder was weighed, extracted with 80% acetone (20 ml), homogenized, centrifuged at 8000 rpm for 15 minutes, and filtered with Whatman paper no. 1 and 42. The filtrate obtained was

added with 80% acetone until the volume reached 25 ml in a volumetric flask. The extract obtained was measured for absorbance at 663 and 645 nm wavelengths using a UV-Vis spectrophotometer. The total chlorophyll content is determined by equation 1.

$$Total\ Chorophyll\ Content\ \left(\frac{mg}{g}WB\right) = (20{,}2A_{663} + 8{,}02A_{645})\ X\ FP/1000 \quad ...(1)$$

A663 means absorbance at 663 and A_{645} means absorbance at 645, then FP means dilution factor.

Zn Content

The sambiloto simplicia powder was weighed (2 g) and ashed at a temperature 600 °C for an hour. After ashing, it was dissolved in 10 ml of 6 N HCl, and then the solution was evaporated by heating in an acid chamber until dry. The residue was dissolved in 0.1 N HNO_3 5 ml and then analyzed by atomic absorption spectrophotometer (AAS, Contr, AA300, Analytic Jena).

Total Phenolic

Total phenolic content (TPC) was determined by the Folin Ciocalteu method[13] used gallic acid as a standard to determine TPC. Acetone extract obtained from analyzing chlorophyll levels was also used to analyze phenolic and flavonoid groups. Sample 50 µl, add 250 µl of Folin-Ciocalteu solution, then leave for 1 minute and add 750 µl of 20% $NaCO_3$, then homogenized with a vortex and distilled water to a volume of 5 ml. After incubation for 5 minutes at room temperature, the absorbance was measured at λ 760 nm. Gallic acid was used as a standard, and a calibration curve was prepared with gallic acid. The TPC calculation result is mg Gallic Acid Equivalent (EAG) per one gram of dry extract.

Total Flavonoid Content

Total flavonoid levels (TFC) were determined using the Wariyah and Riyanto (2020) method. 50 µl extract plus 4 ml distilled water and 0.3 ml 10% $NaNO_2$. After leaving it for 6 minutes, add 0.3 ml of 10% $AlCl_3.6H_2O$, leave it for 5 minutes, then add 4 ml of 10% NaOH. Next, distilled water was added (until the total volume was 10 ml), homogenized with a vortex for 1 minute, and left for 15 minutes. Absorbance was measured at a wavelength of 510 nm. The blank used is distilled water. TFC was calculated using a quercetin standard with a concentration of 1.25-80 mg/L and calculated as mg quercetin equivalent (EK)/g dry based.

Androghapolide Content (AP)

Determination of andrographolide content (APC) using Thin Layer Chromatography (TLC). Using a Microliter syringe, the samples were spotted 1 cm from the bottom with an 8 cm elusion distance on pre-coated silica gel aluminum plate 60F-254 (10 x 10 cm) (E. Merck, Germany) and a Linomat 3 sample applicator. The isolated andrographolide was then subjected to TLC analysis with the reference standard of andrographolide, where a mixture of chloroform and methanol at a ratio of 9:1 was utilized. To ensure the purity of andrographolide, three different mobile phases, including ethyl acetate and acetone 7:3, chloroform and methanol 9:1, and chloroform and acetone 7:3 were used.[13]

Antioxidant Activity

Antioxidant activity was determined with lipid peroxidation inhibition (LPI) and radical scavenging activity (RSA). Determination of lipid peroxidation inhibition (LPI) activity using a linoleic acid emulsion system and the FTC method Suryani *et al*., 2018.[28] The sambiloto extract was made by weighing a 0.5 g sample and adding 10 ml of 80% methanol, homogenizing, filtering using Whatman filter paper no. 42, and adjusting the volume to reach 25 ml. Methanol extract of sambiloto simplicia powder or standard BHT (33.3 µg/ml) was mixed with linoleic acid emulsion made by 1 ml of 2.5% linoleic acid mixed with 0.1 ml of Tween 20. A 4 ml of sambiloto simplicia extract sample and 0.02 M potassium phosphate buffer was added to reach a volume of 10 ml. The emulsion was incubated at 37 ± 10 C in the dark for six days, and every day, a 0.1 ml aliquot was taken for analysis. The degree of oxidation was measured by 0.1 ml aliquot in 5 mL ethanol (75% v/v), 0.1 mL ammonium thiocyanate (30% w/v), and 0.1 mL $FeCl_2$ (0.02 M in 3.5% HCl v/v), then homogenized. After homogenization, the absorbance was measured at 500 nm and repeated thrice. The solution without extract was used as a blank, and BHT was used as a comparison at the same concentration of 100 ppm. The percentage of inhibition of lipid peroxidation was determined using equation 2 with Ao absorbance of the control and A1 absorbance of the sample.

$$LPI(\%) = 100 - [(A_1 / A_0)X100] \quad ...(2)$$

The DPPH free radical scavenging activity (RSA) was determined using the method of Kang *et al*. (2018).[15] This method was chosen because the sample volume tested was smaller than the volume of the DPPH solution, so the effect of chlorophyll color during measurements could be minimized. A sample of 0.2 ml methanol extract of sambiloto simplicia powder, added to 3.8 ml of 0.1 mM DPPH solution in methanol solvent, was mixed using a vortex for 1 minute; the resulting filtrate was incubated in the dark a room temperature for 30 minutes. Controls were made using methanol as a sample substitute and Butylated Hydroxytoluene (BHT) as a comparison at a concentration of 100 ppm. After incubation, the filtrate was measured for absorbance using UV-Vis spectrophotometry (Shimadzu) at a wavelength of 515 nm. The data obtained are A_0: absorbance of DPPH without sample, A_S: absorbance of model added with DPPH, and A_b: absorbance of extract sample without DPPH. Radical Scavenging Activity is expressed in percentage. The RSA value shows the ability of the sample to bleach DPPH violet and is calculated using Equation 3 (Kang *et al*., 2018).[15]

$$RSA(\%) = [A_0 - (A_S - A_b)/A_0)X100 \quad ...(3)$$

Data Analysis

The research data was analyzed with ANOVA and the Pearson correlation test with the SPSS software. The data was obtained from two treatment batches and three replicate analyses.

Results

TCC and Zn Content

Figure 1 (a) shows that the high ZnCl2 concentration promotes the high number of TCC. However, the effect is insignificant if the heating oven is more than 200 ppm. When heating with an autoclave at more than 300 ppm, the chlorophyll content was decreased because the residual chlorine causes chlorophyll oxidation.[16]

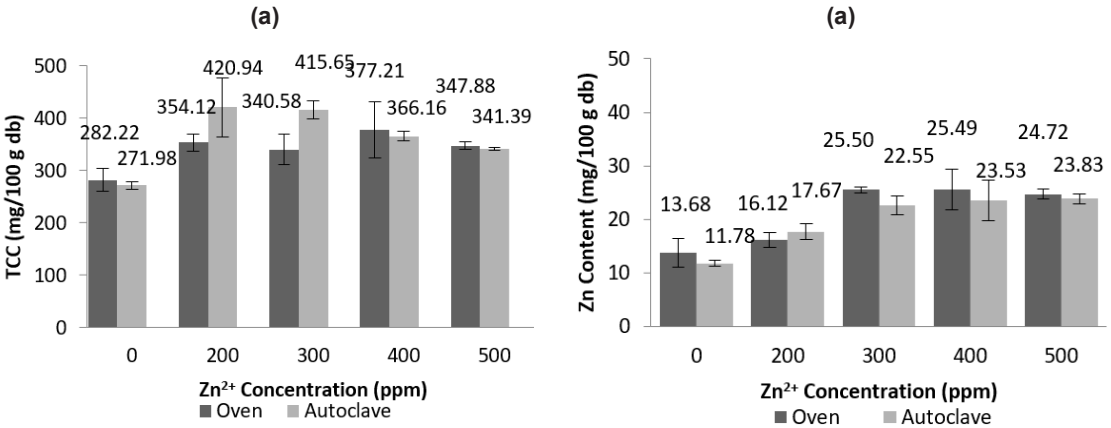

Fig. 1: TCC (a) and Zn content (b) of sambiloto simplicia powder in various heating methods and Zn concentrations in the formation of the Zn-chlorophyll complex

Table 1. Correlation between TPC, TFC, APC, Zn content, and TCC on the antioxidant activity of sambiloto simplicia powder

Antioxidant activity	TPC	TFC	AP Content	Zn Content	TCC
LPI	0.224* (0.045)	-0.167 (0.203)	0.547** (0.001)	-0.390** (0.002)	-0.025 (0.85)
RSA	0.498** (0.001)	0.683** (0.001)	0.239* (0.049)	0.635** (0.001)	0.424** (0.001)

Note: Value in blanket means p value
*Significant in P<0.05
**Highly Significant in P<0.01.

Androghapolide Content (AP), Total Phenolic Content (TPC), and Total Flavonoid Content (TFC)

Figure 2 (a) shows that AP content increased with increasing ZnCl2 concentrations. However, in the oven heating method, it is insignificant, whereas in the autoclave, it expands to 300 ppm and decreases again at a concentration of 500 ppm. Autoclave heating can better maintain TPC and TFC as with chlorophyll and andrographolide content. This is because the phenolic and flavonoid components are very susceptible to heat.

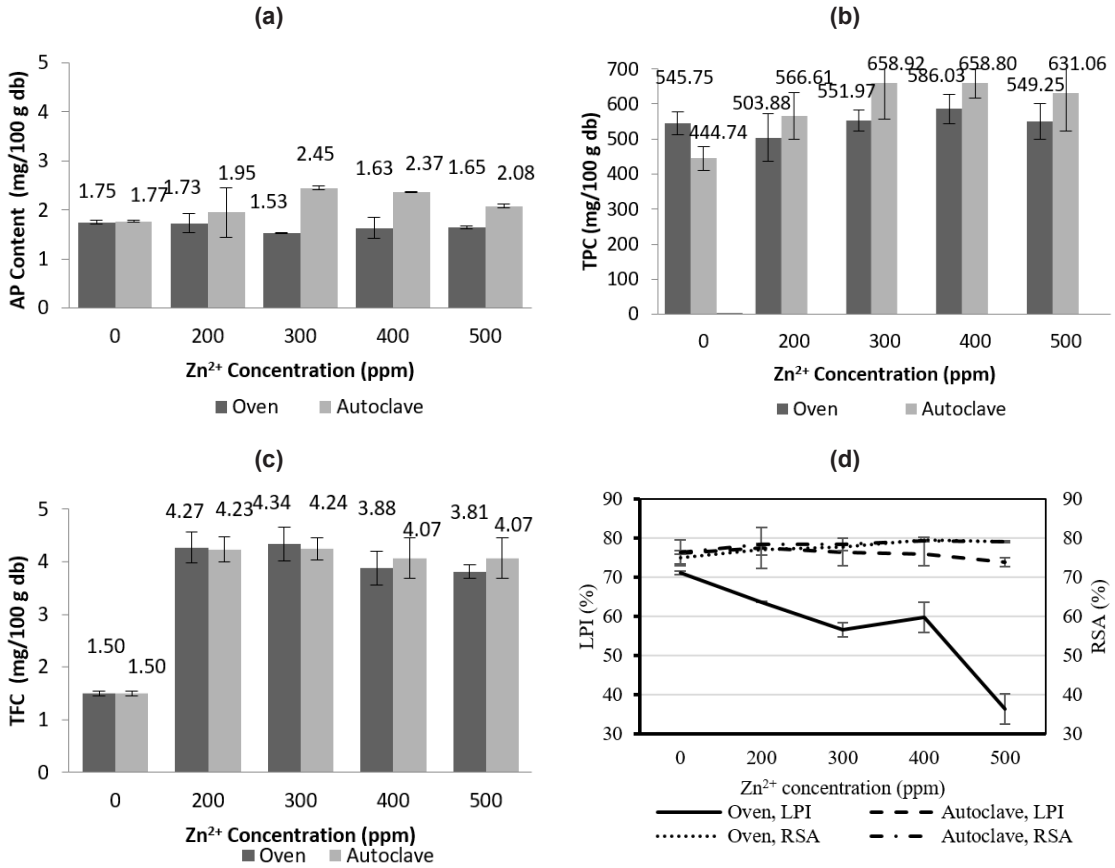

Fig. 2: AP content (a), TPC (b), TFC (c), and antioxidant activity (d) of sambiloto simplicia powder in various heating methods and Zn concentrations in the formation of the Zn-chlorophyll complex

Antioxidant Activity

Figure 2 (d) shows that increasing concentration of Zn^{2+} will be followed by the higher number of LPI when heated in an autoclave. The simplicia sambiloto powder has RSA 36.46-77.52%. The higher the Zn^{2+} concentration, up to 400 ppm, the higher the RSA.

Correlation

Data in Table 1 shows that the lipid peroxidation inhibitory activity of sambiloto simplicia powder is correlated positively with an andrographolide content ($P<0.01$) and negatively with bound Zn levels and associated with total phenolic groups but not associated with total flavonoids and total chlorophyll levels ($P>0.05$). However, it was not related to total flavonoids and total chlorophyll content ($P>0.05$). Meanwhile, DPPH radical scavenging capacity was positively correlated ($P<0.01$) with TPC, TFC, Zn content, and TCC. Moreover, it is positively correlated with andrographolide content ($P<0.05$).

Discussion

TCC and Zn Content

Figure 1 shows that the higher the ZnCl2 concentration, the higher the total chlorophyll

content; however, if the oven is heated with a reagent concentration of more than 200 ppm, the effect of increasing chlorophyll levels is not significant. Meanwhile, when heating with an autoclave, the total chlorophyll level still increases to 300 ppm. At 400 ppm and 500 ppm, the chlorophyll content was decreased. The chlorine residue is suspected to be more significant, resulting in chlorophyll oxidation, which reduces chlorophyll content.[16] On heating with an oven, the formation of metallochlorophyll complexes is ineffective. The heat conductor medium in oven heating is dry air so that the reaction could be more optimal. Higher $ZnCl_2$ concentrations will result in Cl_2 residues triggering chlorophyll degradation, which has not yet formed a Zn-chlorophyll complex, so chlorophyll levels are lower.[9]

Data in Figure 1 (b) shows that the higher the $ZnCl_2$ concentration in oven and autoclave heating, the higher the Zn content, but it is insignificant in oven heating with more than 300 ppm. The higher the concentration of $ZnCl_2$, the more Zn is bound by chlorophyll and other components such as phenolics and flavonoids. Zn binding is less effective in oven heating because medium heating is dry air, whereas autoclave heating is under water vapor pressure; thus, damage in oven heating is very high. Elshaafi *et al*.[17] state that increasing the oven heating temperature by 10 °C has resulted in high levels of phenolic and flavonoid component damage. The Zn binding ability of the sambiloto simplicia powder is theoretically higher than the Zn critical ability of the chlorophyll molecules[18] in the sambiloto simplicia powder. This is caused by other components that can also bind to Zn^{2+}, namely aromatic compounds such as 2-acetyl-1-pyrroline,[19] phenolics,[20] and flavonoids.[21]

AP content, TPC, and TFC

An essential component in sambiloto is andrographolide, and this compound plays many roles such as an antioxidant,[3] anti-inflammatory,[22] hepatoprotective,[23] anticancer, antiviral, antihyperglycemic, antibacterial, and antifertility.[24] Figure 2 (a) showed that the interaction between varied $ZnCl_2$ concentration and heating methods significantly affected the AP content of sambiloto simplicia powder. As in the results of the TCC analysis, AP content also increased in line with increasing $ZnCl_2$ concentrations. However, in the oven heating method, it is insignificant, whereas in the autoclave, it expands to 300 ppm and decreases again at a concentration of 500 ppm. According to Chokthaweepanich *et al*.,[25] the optimum temperature for drying sambiloto in a hot air oven that retains andrographolide is 65 °C. However, in this study, at a temperature of 110°C with autoclave heating, it could prevent the degradation of andrographolide in Simplicia Sambiloto powder. Figure 2 (b) shows that the autoclave heating method can better maintain phenolic levels as with chlorophyll and andrographolide content. This is because the phenolic component is very susceptible to heat. Kessy *et al*.[2] state that steam blanching can maintain phenolic components compared to oven heating. The results of total flavonoid levels also had the same trend as total phenolic levels. The data from Figure 2 (c) show that the autoclave heating method can better maintain total flavonoid levels. At the same temperature (110 °C), in autoclave heating, the stability of flavonoids is reported to be higher than oven heating.[26]

Antioxidant activity

Figure 2 (d) shows that increasing concentration of Zn^{2+} contributes the higher LPI when heated in an autoclave. This is because the total phenolic and flavonoid content, andrographolide, and chlorophyll content of sambiloto simplicia powder with oven heating tend to be smaller, and Zn content is higher. The effectiveness of inhibiting linoleic acid peroxidation from chlorophyll extract is also influenced by the presence of phenolic and flavonoid components and total carotenoids in the chlorophyll extract.[27] Sheeja *et al*.[28] also stated that andrographolide extract has lipid peroxidation inhibitory activity of up to 80%. The LPI was compared with BHT, and it was found that simplicia sambiloto powder had an LPI reaching 97.56% of BHT.

DPPH radical scavenging activity is one of the most widely used methods to measure the antioxidant activity of a material because it is relatively simple and fast, with results that can be compared with standards. The ability to capture DPPH radicals is influenced by the molecular structure of chlorophyll and the presence of phenolic, flavonoid, and andrographolide compounds. Figure 2 (c) shows that simplicia sambiloto powder has RSA 36.46-77.52%. The higher the Zn^{2+} concentration, up to 400 ppm, the higher the RSA. This is related to increased

TCC, TPC, TFC, and AP content. Previous research discovered that blanching fresh sambiloto leaves in a Zn solution could increase the antioxidant activity of the sambiloto simplicia powder.[11] Adam *et al*.[3] stated that androghapolide extract has moderate DPPH radical scavenging power with an IC50 of 0.883 ± 1.597 mg/ml and 0.514 ± 0.285 mg/ml compared to vitamin C 0.048 ± 0.004 mg/ml. In this study, the DPPH radical capture capacity was compared with BHT. The RSA of simplicia sambiloto powder can reach 94.25% compared to BHT.

Correlation

Data in Table 1 shows that the lipid peroxidation inhibitory activity of sambiloto simplicia powder is positively correlated with an andrographolide content($P<0.01$) and correlated negatively with bound Zn content ($P<0.01$) and associated with total phenolic content but not related to total flavonoids and total chlorophyll content ($P>0.05$). Meanwhile, DPPH radical scavenging capacity was highly positively correlated ($P<0.01$) with TPC, TFC, Zn content, and TCC and positively correlated with andrographolide content ($P<0.05$). TCC was not associated with LPI because the higher levels of Zn in the material can act as a cofactor for lipid oxidation. However, Zn is a cofactor for enzymes that act as antioxidants in the body, so Zinc deficiency can result in reduced cell immunity, which can cause inflammation and cell death.[29] Therefore, it is essential to develop food or drink products rich in Zinc that can increase zinc intake to meet the body's needs.

Conclusion

Based on the research results, it can be concluded that the concentration of $ZnCl_2$ as a reagent for the formation of Zn-chlorophyll and the heating method has a significant effect on chlorophyll content, Zn content, total phenolic content, total flavonoid content, andrographolide content and antioxidant activity of sambiloto simplicia powder. The higher the concentration of $ZnCl_2$ up to 300 ppm, the greater the chlorophyll content, phenolic and flavonoid content, Zn content, and andrographolide content.

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Conflict of Interest

The author(s) declares no conflict of interest.

Author Contributions

Chatarina Lilis Suryani and FX Suwarta conceived and designed the experiments; Ichlasia Ainul Fitri performed the experiments and analyzed the data; Chatarina Lilis Suryani contributed reagents/ materials/analysis tools; Chatarina Lilis Suryani wrote the initial draft; Ichlasia Ainul Fitri and FX Suwarta revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Not applicable

Ethic Approval Statement

Not applicable

References

1. Adam R., Ramli F., Hamid M., Wahab R. A. Antioxidant Activity of Andrographolide from Andrographis paniculata leaf and Its Extraction Optimization using Accelerated Solvent Extraction *J. of Tropical Life Science.* 2023; 3 (1): 157–170 .https://doi.org/10.11594/jtls.13.01.16
2. Kessy H. N. E., Hu Z., Zhao L., Zhou M. Effect of steam blanching and drying on phenolic compounds of *litchi pericarp Molecules.* 2016; 21(6): 1-9. https://doi.org/10.3390/molecules21060729
3. Rababah T. M., Al-U' D. M., Alhamad M., Al-Mahasneh M., Ereifej K., Andrade J., Altarifi B., Almajwal A., Yang W. Effects of drying process on total phenolics, antioxidant activity and flavonoid contents of common Mediterranean herbs *International Journal of Agricultural and*

Biological Engineering. 2015; 8(2): 145–150. https://doi.org/10.3965/j.ijabe. 20150802.1496

4. Isleroglu H., Sakin-Yilmazer M., Kemerli-Kalbaran T., Uren A., Kaymak-Ertekin F. Kinetics of color, chlorophyll, and ascorbic acid content in spinach baked in different types of oven *International Journal of Food Properties.* 2017; 20(1): 2456–2465 https:doi.org/10.1080/10942912.2016. 1240689
5. Chokthaweepanich H., Chumnanka C., Srichaijaroonpong S., Boonpawa R. Effect of harvesting age and drying condition on andrographolide content, antioxidant capacity, and antibacterial activity in Andrographis paniculata *(Burm.f.) Nees AIMS Agriculture and Food.* 2023; 8(1): 137–150. https://doi.org/10.3934/AGRFOOD.2023007
6. Ngo T., hao Y. Formation of zinc-chlorophyll-derivative complexes in thermally processed green pears *(Pyrus communis L.) Journal of Food Science.* 2017; 72(1): 397–404. https://doi.org/10.1111/j.1750-3841.2007.00465.x
7. Usman., Fitri I. A., Suryani C. L. Pengaruh Jenis Medium Sumber Zn^{2+} dan Lama Blanching Terhadap Aktivitas Antioksidan Bubuk Simplisia Sambiloto (*Andrographis Paniculata*) Effect of Medium Type of Zn^{2+} Source and Blanching Time on Antioxidant Activity of Sambiloto (*Andrographis Panicula. Prosiding Seminar Mini Riset Mahasiswa*. 2022; 1: 54–66.
8. Akther S., Jothi J. S., Badsha M. R., Rahman M. M., Das G. B., Alim M. A. Drying methods effect on bioactive compounds, phenolic profile, and antioxidant capacity of mango powder. *Journal of King Saud University – Science.* 2023. 35(1): 102-370 . https://doi.org/10.1016/j.jksus.2022.102370
9. Suryani C. L., Fitri I. A., Evlin E., Suwarta F. X .The Effects of Metallochlorophyll Formation and Pretreatment on Color, Chlorophyll Content, Total Phenolic Content, and Antioxidant Activity of Sambiloto *(Andrographis paniculata) Simplicia Powder.* 2023; 11(2): 266–274. https://doi.org/10.13189/ujar.2023.110205
10. Suryani C. L., Wahyuningsih T. D., Supriyadi., Santoso U. The potential of mature pandan leaves as a source of chlorophyll for natural food colorants *Jurnal Teknologi Dan Industri Pangan.* 2020; 31(2): 127–137. https://doi.org/10.6066/jtip.2020.31.2.127
11. Wang W., Li J., Zhang H., Wang X., Fan J., Zhang X. Phenolic compounds and bioactivity evaluation of aqueous and methanol extracts of *Allium mongolicum Regel Food Science and Nutrition.* 2020; 7: 779–787. https://doi.org/10.1002/fsn3.926
12. Wariyah C., Riyanto . Hypoglycemic Activity of Aloe vera Powder and Gel Drink in Alloxan-induced Diabetic Rats *Research Journal of Medicinal Plants.* 2020; 14(3): 149–155 . https://doi.org/10.3923/rjmp.2020.149.155
13. Sajeeb B. K., Kumar U., Halder S., Bachar S. C. Identification and quantification of andrographolide from andrographis paniculata (Burm. F.) wall. ex nees by RP-HPLC method and standardization of its market preparations Dhaka University *Journal of Pharmaceutical Sciences.* 2015; 14(1): 71–78. https://doi.org/10.3329/dujps.v14i1.23738
14. Suryani C. L., Wahyuningsih T., Supriyadi., Santoso U . Derivatization of chlorophyll from pandan (*Pandanus amaryllifolius Roxb*.) leaves and their antioxidant activity. *Periódico Tchê Química.* 2020; 17(36): 1110–1126.
15. Kang Y. R., Park J., Jung S, K., Chang Y. H. Synthesis, characterization, and functional properties of chlorophylls, pheophytins, and Zn-pheophytins *Food Chemistry.* 2018; 245(1): 943–950. https://doi.org/10.1016/j.foodchem.2017.11.079
16. Ebenezer V., Nancharaiah Y. V ., Venugopalan V. P. Chlorination-induced cellular damage and recovery in *marine microalga, Chlorella salina Chemosphere.* 2012; 89(9): 1042–1047. https://doi.org/10.1016/j.chemosphere.2012.05.067
17. Elshaafi I. M., Musa K. H., Sani A. Effect of oven and freeze drying on antioxidant activity, total phenolic and total flavonoid contents of fig *(Ficus carica L.) leaves. Food Research*. 2020; 4(6): 2114–2121. https://doi.org/10.26656/fr.2017.4(6).072
18. Bohn T., Walczyk T., Leisibach S., Hurrel R. F. Chlorophyll-bound magnesium in commonly consumed vegetables and fruits : *Relevance to magnesium nutrition Journal of Food Science.* 2004. 69(1): 347–350.
19. Fang M. C. Synthesis and stabilization of selected heterocyclic aroma compounds. Dissertation University of Illinois at Urbana-Champaign. 2013.

20. Wang T., Jónsdóttir R., Ólafsdóttir G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chemistry.* 2009; 116(1): 240–248 https://doi.org/10.1016/j.foodchem.2009.02.041
21. Cherrak S. A., Mokhtari-soulimane N., Berroukeche F., Merzouk H., Elhabiri M., Bensenane B., Cherbonnel A., Merzouk H., Elhabiri M. *In vitro* antioxidant versus metal ion chelating properties of flavonoids: A structure-activity investigation PLoS ONE. 2016; 11(1): 1–21 https://doi.org/10.1371/journal.pone.0165575
22. Silalahi M. Sambiroto (*Andrographis paniculata*) dan *Bioaktivitasnya BEST Journal (Biology Education, Sains and Technology).* 2020. 3(1): 76–84 https://doi.org/10.30743/best.v3i1.2448
23. Ali S. K., Makeen H. A., Khuwaja G., Alhazmi H. A,. Sharma M., Koty A., Mazahirul I., Parveen H., Mohammed A., Mukhtar S., Alam M. F. Assessment of the Phytochemical Profile, Antioxidant Capacity, and Hepatoprotective Effect of Andrographis paniculata against CCl4-Induced Liver Dysfunction in Wistar Albino Rats. *Medicina.* 2023; 59(7): 1-16. https://doi.org/10.3390/medicina59071260
24. *Vetvicka V., Vannucci L*. Biological properties of andrographolide, an active ingredient of *Andrographis Paniculata*: a narrative review *Annals of Translational Medicine*. 2021; 9(14): 1186–1186. https://doi.org/10.21037/atm-20-7830
25. Chokthaweepanich H., Chumnanka C., Srichaijaroonpong S., Boonpawa R. Effect of harvesting age and drying condition on andrographolide content, antioxidant capacity, and antibacterial activity in Andrographis paniculata (*Burm.f.*) *Nees AIMS Agriculture and Food.* 2023; 8(1): 137–150 https://doi.org/10.3934/AGRFOOD.2023007
26. Ioannou I,. Chekir L., Ghoul M. Effect of heat treatment and light exposure on the antioxidant activity of flavonoids Processes. 2020; 1078(8): 1-17. https://doi.org/10.3390/pr8091078
27. Suryani C. L., Tamaroh S., Budipitojo T. Increased of hypoglycemic effect and pancreatic regeneration of Pandanus amaryfollius leaves ethyl acetate extract in streptozotocin-induced diabetic rats *International Food Research Journal.* 2018; 25: 1792–1798 http://www.ifrj.upm.edu.my
28. Sheeja K., Shihab P. K., Kuttan G. Antioxidant and anti-inflammatory activities of the plant Andrographis paniculata nees Immunopharmacology and Immunotoxicology. 2006; 28 (3): 129–140. https://doi.org/10.1080/08923970600626007
29. Maares M., Haase H. Zinc and immunity: *An essential interrelation Archives of Biochemistry and Biophysics*. 2016; 611(1): 58–65. https://doi.org/10.1016/j.abb.2016.03.022