

Combination of polyherbal *Phyllanthus reticulatus* with *Zingiber officinale* and *Cymbopogon citratus* to optimize the antioxidant capacity

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ABSTRACT

Currently, the public is interested in polyherbal-based foods and beverages as a source of natural antioxidants. The aim of the study is to evaluate the antioxidant properties and the phenolic and flavonoid compounds of formulations containing *Z. officinale*, *C. citratus*, and *P. reticulatus* (ZCP). Each sample was extracted using the maceration process in an ethanol solvent at room temperature for three 72-hour periods. There were fourteenth formulation of *Z. officinale* rhizome, *C. citratus* leaves, and *P. reticulatus* fruit which used Design of Expert (DoE). The DPPH method was used to determine the power of antioxidants. The flavonoid content of the extract was measured using the colorimetric method and $AlCl_3$ reagent, while phenolics content using Folin-Ciocalteu. The formulations ZCP 1:0:0, 0:0:1, and 1:1:1 showed the antioxidant capacity in a strong categorization, with an IC_{50} value less than 50 $\mu g/ml$, while ZCP 0:1:0 was in a weak categorization ($IC_{50} > 250 \mu g/mL$). Another ZCP formulation was in a medium category. The ZCP 1:1:1 formulation was suggested as the best one for this investigation, which contains three plant samples. This formulation is interesting for further toxicity studies and in vivo testing so that it can be applied as an antioxidant-rich supplement product.

Keywords: antioxidant capacity, polyherbal formulation, *Zingiber officinale*, *Cymbopogon citratus*, *Phyllanthus reticulatus*

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INTRODUCTION

The natural world has historically been a fantastic source of therapeutic substances, offering a wide range of medicinal plants that produce useful phytochemicals (Foghis et al., 2023). According to WHO, traditional medicine is defined as a number of health behaviors, techniques, information, and traditions that include manual methods, physical activity, religious treatments, and substances derived from organisms such as animals, plants, or mineral content that are used singly or in combination to encourage health and prevent disease (Mukherjee & Karati, 2023). One function that is currently being studied a lot is antioxidant capacity which is closely related to the body's immunity.

Antioxidant compounds are beneficial for maintaining a healthy body because they are able to maintain a balance between oxidation and anti-oxidation in the body, preventing oxidation in the body which can trigger various diseases (Wang et al., 2014; Sindhi et al., 2013). Several secondary metabolites were reported to have antioxidant abilities such as polyphenols (phenolic acids, flavonoids, anthocyanins, lignin, and stibine), carotenoids (xanthophylls and carotene), and ascorbic acid, tocopherol, and also tannin (Irfan et al., 2022; Baiano & Nobile, 2016; Xu et al., 2017; Wafa et al., 2016). Fruit and vegetable are known as sources of exogenous antioxidants because of their antioxidant compounds contents (Bouayed & Bohn, 2010). Since the time of their forebears, the community has used ginger (*Zingiber officinale*) and lemongrass (*Cymbopogon citratus*) as components of herbal medicine and traditional medicine. Several scientists (Begum et al., 2006; Jamal et al., 2008) have studied Mangsian, the Indonesian name for *Phyllanthus reticulatus*, but the people there is yet to utilize it as a source of antioxidants.

A lot of people think that preparations from multiple plants are only useful as medicines. While many polyherbal extracts still need to be researched, some have been scientifically shown to be effective in the treatment of oxidation stress-related diseases (Adejoh et al., 2016). The existence of many antioxidant molecules in raw materials can have interactions with each other that might be additive, antagonistic, or synergistic (Gupta et al., 2021; Sindhi et al., 2013; Bouayed & Bohn, 2010). Positive interactions, sometimes referred to as synergistic interactions, occur when two or more substances are combined and the result exhibits a greater action than the total number of chemicals. Researchers alike use the concept of synergism in their research on antioxidants, antimicrobials, antifungals, and novel therapeutic formulation (Blesson et al., 2015). On the other hand, antagonism is a result that is diminished when combined. Findings that are both positive nor negative are characterized as being indifferent, and the whole effect of the combination is additive.

Several studies in vitro and in vivo have documented antioxidant properties of *Z. officinale*, *C. citratus*, and *P. reticulatus* either by itself or in combination with other plants. Numerous investigations showed the phytopharmaceutical properties of ginger extract under specific circumstances, including considerable superoxide radical scavenging action, reducing stresses and damage to cells and anti-inflammation (Mustafa & Chin, 2023; Rostamkhani et al., 2022; Adejoh et al., 2016; Morakinyo et al., 2012). The aqueous and ethanol extracts of *Z. officinale* from Nigerian may be useful in the treatment of conditions oxidative stress-related diseases like atherosclerosis and diabetes mellitus (Morakinyo et al., 2012). The study of drying methods on ginger's antioxidant found that water-ethanol mixtures are effective for extracting polar antioxidants whereas sun-dried ginger showed the highest recovery of phenolic compounds contents (Mustafa & Chin, 2023). In the study of lemongrass leaves, extraction using the maceration technique showed that the 70% ethanolic concentration had the highest levels of scavenging activity compared to the 50% ethanolic concentration and the 70% acetone concentration (Irfan et al., 2022). (Wuryatmo et al., 2021) stated that lemongrass has the potential to be employed as a natural food preservative, especially in high fat food products, as evidenced by its high antioxidant activity. Its study showed that the ethanol extract of stem *C. citratus*, total phenolic content of 19.31 mg GAE/g and flavonoids at 3.31 mg GAE/g. This outcome was connected to the extract's 79.96% antioxidant activity. At about mangsian (*P. reticulatus*), although that plant has been researched by some researchers (Maruthappan & Shree, 2010; Begum et al., 2006), the general public has not yet exploited it as a source of antioxidants. Many researchers studied about the combination polyherbal about ginger, lemongrass, and mangsian. The combination of ginger and sappan wood also increases

antioxidant activity better than being separated reported (Widyapuspa et al., 2022). The analgesic and anti-inflammatory activity of a combination of *Phyllanthus reticulatus* and *Mimosa pigra* (Akhter et al., 2018). A study of the three species of *Z. officinale* with *Cinnamomum burmannii* and *Caesalpinia sappan* showed that the combination of ginger had either higher total phenolic or antioxidant activity than the non-combination (Mahmudati et al., 2022). Study on a combination of herbs, named Trikatuk, in many combinations, resulting in most of the combinations investigated in the present study also exhibiting additive or synergistic effects (Nutmakul & Chewchinda, 2023).

The research of the relationship between the antioxidant properties and antioxidant components of blends of *Z. officinale*, *C. citratus*, and *P. reticulatus* has not yet been reported. The time difference from standard practice has not been determined by other investigations. Finding the best formulations for health supplements, especially those that are providers of antioxidants, through the research of polyherbal is interesting. So, the purpose of this study is to develop a composition containing *Z. officinale* rhizome, *C. citratus* leaves, and *P. reticulatus* fruits and to evaluate the antioxidant properties and their phenolic and flavonoid compounds.

MATERIALS AND METHOD

Materials

The design of research using method of experimental design. The plant samples were included with *Zingiber officinale*, *Cymbopogon citratus*, and *Phyllanthus reticulatus*. The plants collection (No. 001 until No. 004/2014/FPBUKSW/Koleksi) were preserved in *Biologi Dasar* Laboratory, Faculty of Biology, Satya Wacana Christian University, Salatiga, Indonesia. The parts of the plant used are the rhizome of *Z. officinale*, the leaf of *C. citratus*, and the fruit of *P. reticulatus*.

Methods

Extract and formulation preparation

Every research material (Figure 1) was prepared as 5 pieces as a replicate of the study. With the exception of *P. reticulatus*, every material was broken up into tiny pieces and air dried before being baked at 50°C. The dry sample was then counted using a mixer. Using the maceration procedure, ethanol (1: 1.5 w/v) was used as the solvent to create the extracts. Three times of macerations, duration of 72 hours each of them was performed. After each maceration process, the macerate is filtered, and the supernatant is then subjected to another maceration. The mixed filtrate was dried at 40°C in a Rotavapor R114 Buchi rotary evaporator under vacuum (Eyela A-1000S).

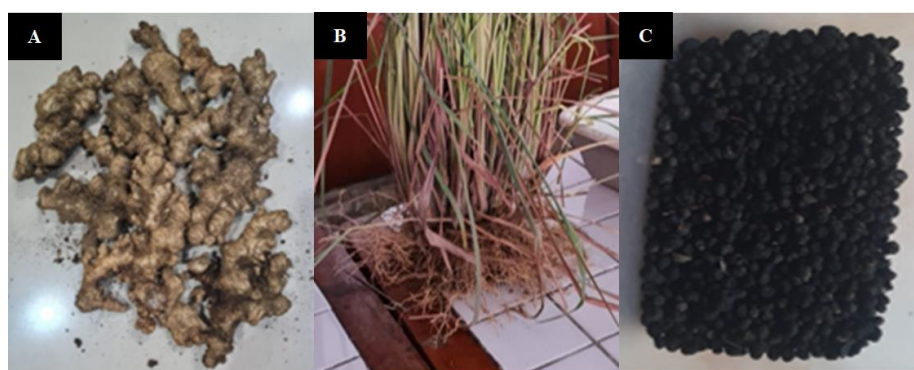


Figure 1. The samples of research. (A) Rhizome of *Z. officinale* (B) Leaves of *C. citratus*, (C) Fruit of *P. reticulatus*

The compositions of extract were designed using the Desain of Experiment was used to create the extract formulation. (DoE) (Table 1).

Table 1. Composition of polyherbal, a mixture of ethanol extracts of ginger rhizome (*Z. officinale*), lemongrass leaves (*C. citratus*), and mangsian fruit (*P. reticulatus*) using Design of Expert (DoE) (Design-Expert 13.0 trial version)

Polyherbal formulation	Percentage of composition (%)		
	<i>Z. officinale</i>	<i>C. citratus</i>	<i>P. reticulatus</i>
ZCP 1:0:0	100.00	0.00	0.00
ZCP 1:1:4	16.67	16.67	66.67
ZCP 1:4:1	16.67	66.67	16.67
ZCP 1:0:1	50.00	0.00	50.00
ZCP 0:0:1	0.00	0.00	100.00
ZCP 0:1:0	0.00	100.00	0.00
ZCP 1:1:0	50.00	50.00	0.00
ZCP 4:1:1	66.67	16.67	16.67
ZCP 1:0:0	100.00	0.00	0.00
ZCP 1:1:0	50.00	50.00	0.00
ZCP 0:1:0	0.00	100.00	0.00
ZCP 0:0:1	0.00	0.00	100.00
ZCP 0:1:1	0.00	50.00	50.00
ZCP 1:1:1	33.33	33.33	33.33

Antioxidant activity assay

The ability of antioxidants was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (Senja et al., 2016) with slight modification. The solvent used is methanol. Sample concentration series for the determination of IC₅₀ values in the range of 50, 100, 150, and 200 µg/mL. At amount of 1 mL of sample was added with 1 mL of DPPH, without adding ethanol up to 5 mL. The time incubation of the mixture was 30 minutes in the dark condition. In the measurement of absorbance at wavelength of 517 nm, a Shimadzu UV mini1240 UV -Visible spectrophotometer was used.

Phenolic content assay

Using the Folin-Ciocalteu reagent with a colorimetric technique, the extract's total phenolic acid content was determined Almey et al. (2010) with slight modification. Applying gallic acid as a standard phenolic compound in concentration series 20, 40, 60, 80, and 100 µg/mL. The extract concentration analyzed was 100 µg/mL. The spectrophotometer used for mixture absorbance measurement is UV-Visible spectrophotometer (Shimadzu UV mini1240), at a wavelength of 550 nm. Phenolic acid content (mg GAE/g extract) = c (V/m). c: gallic acid concentration based on gallic acid linear regression equation (mg/l); V = extract volume (l); m = mass of extract (g).

Flavonoid content assay

The colorimetric technique with AlCl₃ reagent were used to determine the extract's flavonoid content (John et al., 2014) with slight modification. Absorbance measurement of the sample was carried out at a wavelength of 415 nm using UV-Visible spectrophotometer (Shimadzu UV mini1240), at a wavelength of 415 nm. As a reference substance, quercetin was utilized at concentrations of 20, 40, 60, 80, and 100 mg/l. Flavonoid content (mg QE/g extract) = c (V/m). c: flavonoid concentration based on quercetin linear regression equation (mg/l); V = extract volume (l); m = mass of extract (g).

Data Analysis

The data were analyzed statistically using the analysis of variance test to determine the significant difference in values between extracts and test parameters and continued with the Tukey test to determine the correlation between the concentration of the test compound and the antioxidant ability of the sample.

RESULT AND DISCUSSION

Antioxidant activity

The assay of the antioxidant ability of each formulation was done using the DPPH method. The test using DPPH is a non-enzymatic in vitro test that is widely used to measure the antioxidant capacity of a compound. This method is widely used because its implementation is very simple, fast using an ultraviolet-visible spectrophotometer, and low cost (Dontha, 2016). Using the DPPH technique, the inhibitory potential of each formulation was examined at concentrations of 50, 100, 150, and 200 µg/mL. A linear regression equation was developed from the data already collected, and the formulation concentration at 50% inhibition (IC₅₀ value) was calculated (Table 2).

Table 2. The phenolic and flavonoid content, and IC₅₀ values of ethanol extracts of ginger (*Z. officinale*) rhizome, lemongrass (*C. citratus*) leaves, and mangsian (*P. reticulatus*) fruit in various compositions

Polyherbal formulation	Flavonoid content (µg/g extract)	Phenolic content (µg/g extract)	IC ₅₀ value of antioxidant (µg/mL)	Categories of antioxidant activity*
ZCP 1:0:0	13.82 ± 2.30 ^{cde}	18.92 ± 1.01 ^a	64.4 ± 6.1 ^l	Strong
ZCP 1:1:4	16.67 ± 1.57 ^{bcd}	10.79 ± 0.44 ^{ef}	101.8 ± 6.5 ^h	Medium
ZCP 1:4:1	27.18 ± 3.68 ^a	6.52 ± 0.45 ^h	205.8 ± 30.0 ^c	Medium
ZCP 1:0:1	14.13 ± 1.60 ^{cde}	16.02 ± 1.00 ^b	61.4 ± 4.3 ^k	Strong
ZCP 0:0:1	15.75 ± 0.53 ^{bcd}	11.68 ± 0.64 ^{de}	71.6 ± 2.1 ^j	Strong
ZCP 0:1:0	19.68 ± 1.10 ^b	2.08 ± 0.08 ⁱ	396.3 ± 33.0 ^b	Weak
ZCP 1:1:0	27.90 ± 1.24 ^a	9.03 ± 0.57 ^{fg}	150.0 ± 8.2 ^d	Medium
ZCP 4:1:1	12.22 ± 2.59 ^{de}	11.91 ± 0.34 ^{de}	111.2 ± 8.1 ^g	Medium
ZCP 1:0:0	16.28 ± 1.73 ^{bcd}	14.87 ± 0.79 ^{bc}	81.7 ± 4.6 ⁱ	Strong
ZCP 1:1:0	11.32 ± 1.68 ^{ef}	10.66 ± 0.43 ^{ef}	139.6 ± 13.4 ^{de}	Medium
ZCP 0:1:0	20.77 ± 0.36 ^b	2.39 ± 0.09 ⁱ	497.0 ± 74.7 ^a	Weak
ZCP 0:0:1	16.02 ± 0.82 ^{bcd}	13.48 ± 0.75 ^{cd}	63.8 ± 11.8 ^{jk}	Strong
ZCP 0:1:1	18.13 ± 0.72 ^{bc}	8.69 ± 0.42 ^g	116.9 ± 4.4 ^f	Medium
ZCP 1:1:1	7.00 ± 0.00 ^f	11.73 ± 0.52 ^{de}	117.8 ± 5.5 ^f	Medium

Note: Z: *Z. officinale*; C: *C. citratus*; P: *P. reticulatus*. *Based on the IC₅₀ (µg/mL) value. Very Strong= IC₅₀ ≤ 50 µg/mL; Strong = 50 < IC₅₀ ≤ 100; Medium = 100 < IC₅₀ ≤ 250; Weak = 250 < IC₅₀ ≤ 500; Not Active = IC₅₀ > 500 (Molyneux, 2004). The value is the mean ± standard deviations (SD) (n = 3); Duncan's test was used to assess the mean difference between samples (p > 0.05) after one-way ANOVA to determine whether mean values in the same column with different letters differed significantly

Based on the IC₅₀ value, it appears that under single conditions, *Z. officinale* and *P. reticulatus* extracts have strong antioxidant abilities, while *C. citratus* is classified as weak. This shows that mangsian fruit has the potential to be developed as a source of natural antioxidants. When ginger and mangsian are combined 1:1 (Formulation ZCP 1:0:1), the antioxidant activity is still powerful, which is at a strong categorized (IC₅₀ < 100 µg/mL). On the other hand, when the mixture also contains three other types of components, it loses strength and falls into the medium category regardless of how the three ingredients are combined (IC₅₀ value in the range of 101–250 µg/mL).

Exogenous antioxidants mainly come from food and medicinal plants such as fruits, vegetables, flowers, spices and traditional herbal medicine (Bouayed & Bohn, 2010). Consuming fruit and vegetables with antioxidant capacity, as well as medicinal plants and herbs that have these properties can provide optimal health and nutritional outcomes. It is based on quality nutrition that may have beneficial effects in the prevention of some chronic and degenerative diseases such as cancer that are prevalent in society. This study combined ginger rhizome, lemongrass leaves, and mangsian fruits to obtain the combination that has the optimal antioxidant activity.

The ZCP 1:0:0 formulation showed strong antioxidant capabilities with an IC_{50} value $< 100 \mu\text{g/mL}$. The results of the study show that the ethanol extract of *Z. officinale* has a high antioxidant capacity, in accordance with several existing reports. The study by [Mustafa & Chin \(2023\)](#) showed that ethanol extracts had the best free radical scavenging activity with IC_{50} values $< 50 \mu\text{g/mL}$, with IC_{50} values of $15.23 \mu\text{g/mL}$ for sun-dried ginger samples and $22.10 \mu\text{g/mL}$ for oven-drying. The ultrasonic approach produced an extract from ginger that had a very high antioxidant capacity and an IC_{50} value of $51.46 \pm 0.31 \mu\text{g/mL}$ ([Sarastri et al., 2023](#)). There were the same strength categories for antioxidant ability: very strong antioxidant ability. With an IC_{50} of $18.4 \mu\text{g/mL}$, the methanol extracts of *Z. officinale* demonstrated a very strong antioxidant capacity ([Adejoh et al., 2016](#)). The strong antioxidant capacity was also shown in the ZCP 0:0:1 formulation. When compared to ethanolic extracts, *P. reticulatus* whole plant extracts have stronger antioxidant activity ([Maruthappan & Shree, 2010](#)). Antioxidant activity of plant powder of *P. reticulatus* was 82.4%, exhibits good activity when compared to Butylated Hydroxy Toluene (BHT) of 85%, each at a concentration of 400 g/ml. In this study, 200 $\mu\text{g/ml}$ of a fruit extract (ZCP 0:0:1 formulation) had a 99% inhibitory antioxidant. From both studies, it is possible to employ this plant as a powerful source of natural antioxidants, although people have not used this plant much for health. When the two chemicals coexist, they show a certain pattern. When the difference in phenolic and flavonoid concentrations between *Z. officinale* and *P. reticulatus* is 5 $\mu\text{g/ml}$, these conditions provide the extract with strong antioxidant properties, as seen in the ZCP 1:0:0, ZCP 0:0:1, and ZCP 1:0:1 formulation.

Different from *Z. officinale* and *P. reticulatus*, the antioxidant ability of single lemongrass leaves (ZCP 0:1:0 formulation) was classified as weak, with an IC_{50} value of $447 \mu\text{g/mL}$. With a difference of 18 $\mu\text{g/ml}$, this formulation's flavonoid level is significantly higher than its phenolic amount, making it apparent that flavonoids can reduce antioxidant activity. These findings match those of ([Wuryatmo et al. \(2021\)](#)), who discovered that an ethanol extract of lemongrass stems and leaves had an inhibitory activity of 80% and 67%, respectively, at a concentration of 20,000 $\mu\text{g/mL}$. This indicates that it is far less effective as an antioxidant source than ginger and mangsian.

In combination with a greater composition of *C. citratus* (ZCP 1:4:1) and a composition of ginger with the same of *Z. officinale* and *C. citratus* (ZCP 1:1:0), the mixture formed contained 21 $\mu\text{g/mL}$ more flavonoids than phenolics, but the antioxidant capacity was classified as medium. This may occur because the effect of antioxidant compounds in ginger is different from that in lemongrass, even though they are in the same compound group. Although *Z. officinale* and *P. reticulatus* are powerful antioxidants, combined together with *C. citratus*, the antioxidant power is drop to medium capacity. The ZCP 4:1:1 combination further demonstrates the antagonistic nature of the lemongrass presence in the combination; despite the ginger proportion being higher than the lemongrass and mangsian proportions, the mixture's antioxidant capacity only reaches a medium level. [Poh et al. \(2018\)](#) found that the 1:1 ratio of lemongrass-curry leaf extracts, lemongrass-turmeric extract, and lemongrass-ginger extract showed antagonistic interaction effects. ZCP 1:0:0, ZCP 0:0:1, and ZCP 1:0:1 formulation, which components without lemongrass, has an antioxidant capacity that belongs in the high category (IC_{50} were between 60 - 80 $\mu\text{g/mL}$). This demonstrates how the mixture's antioxidant capacity is lowered by the inclusion of lemongrass. As a result, we suggest that formulations ZCP 1:0:0, ZCP 0:0:1, and ZCP 1:0:1 is the best formulation for this investigation.

Bioactive compounds content

The antioxidant capabilities of secondary metabolites, such as polyphenols and anthocyanins, are well documented ([Shi et al., 2018](#); [Lauro et al., 2016](#)). In this study, flavonoids and phenolics were the bioactive substances that were quantified. [Figure 2](#) demonstrates that the flavonoid and phenolic levels were similar throughout the five formulations, including the one that had just *Z. officinale* and *P. reticulatus* and the one that included ginger and mangsian mixed 1:1.

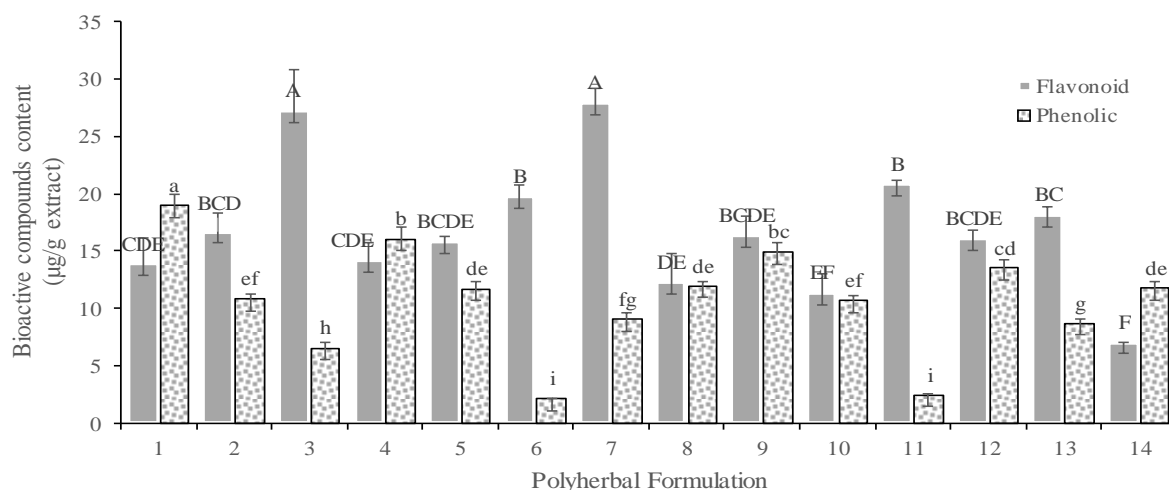


Figure 2. The flavonoids and phenolics content of ethanol extracts of ginger (*Z. officinale*) rhizome, lemongrass (*C. citratus*) leaves, and mangsian (*P. reticulatus*) fruit in various compositions. The composition of ZCP is 1= 1:0:0; 2= 1:1:4; 3= 1:4:1; 4= 1:0:1; 5= 0:0:1; 6= 0:1:0; 7= 1:1:0; 8= 4:1:1; 9= 1:0:0; 10= 1:1:0; 11= 0:1:0; 12= 0:0:1; 13= 0:1:1; 14= 1:1:1. The value is the mean standard deviations (SD) (n = 3); Duncan's test was used to assess the mean difference between samples ($p > 0.05$) after one-way ANOVA to determine whether mean values in the same color of the bar with different letters differed significantly

Researchers have extensively examined flavonoids and phenolic chemicals for their potential as antioxidant sources. A group of chemicals known as phenolic compounds has a variety of structures and phytopharmaceutical properties. Numerous studies' in vitro testing demonstrated that phenolic substances, even at low quantities, have strong antioxidant properties (Kruk et al., 2022). According to Santos-Sánchez et al. (2019), the process by which phenolic compounds exert their antioxidant effect involves the transfer of hydrogen ions from phenol to free radicals, resulting in the establishment of an H-O transition condition. Plants are accessible to a wide variety of flavonoids. Many plants contain a class of chemicals called flavonoids that share the basic structure of polyphenols. Flavanols, flavones, flavonones, isoflavones, flavonols, and flavanonols are the several categories of phenolic chemicals (De Luna et al., 2020). Flavonoids may inhibit the activation of free radicals in four ways that were blocking the activity of nitric-oxide synthase, blocking the activity of xanthine oxidase, altering channel pathways, or interacting with other enzyme systems (Kumar & Pandey, 2013).

All compositions contain phenolics and flavonoids in varying levels (Table 2). Antioxidant ability appears to be strongly influenced by the levels of phenolic compounds. In compositions with strong levels of antioxidant ability (ZCP 1:0:0, ZCP 0:0:1, and ZCP 1:0:1), statistically high levels of phenolics were detected (codes a, b, bc, cd, and de behind the test value) even though the phenolic content at the level is not statistically high (codes bcde and cde behind the test value). On the other hand, one composition at the weak level, namely ZCP 0:1:0, has the lowest phenolic content statistically (code i behind the test value) even though the phenolic content is statistically high (code b behind the test value). Compositions with medium

antioxidant capacity were detected to contain statistically low phenolics even though flavonoid levels were statistically high.

Extraction is a crucial process in this research because it is intended to extract bioactive compounds using a solvent (Le et al., 2018; Othman et al., 2015). In their investigation, Irfan et al. (2022) performed maceration on lemongrass leaves for 24 hours using 50% and 70% ethanol to produce a yield of around 20%. The yield appears to be the same as the study's findings, with a shorter time investment. In contrast to the time needed in this investigation, which was up to 3 x 72 hours, it appears that heating the maceration conditions to a temperature of 40°C can speed up the compound extraction process. The study on *Fortunella polyandra* showed that extraction at a temperature of 30 °C resulted in an extract with optimum antioxidant activity (Elias et al., 2023). Maceration of *Z. officinale* using maceration method done 3 x 24 hours yield the 7.59% of extract (Andriyani et al., 2015). This study's reduced yield value demonstrated how the passage of time affects the outcomes of extraction. The yield obtained increases with longer extraction times.

Oxidation-reduction reactions are a pairs important processes that in occur all the time in the cell. Free radicals are one of caused of oxidation reaction. However, certain oxidation processes become harmful to cells because of its negative effects. Some compounds have the ability to bind free radicals so that oxidation reactions do not occur and prevent damage to healthy cells, known as antioxidants (Dontha, 2016). In assessing the antioxidant ability of natural ingredients, before carrying out the in vivo test, the researchers conducted an in vitro test first. It is assumed that compounds that have high antioxidant abilities in vitro will also have high abilities in vivo (Tukun et al., 2014).

The correlation of phenolic, flavonoid content, and antioxidant activity

The antioxidant ability of a plant extract is influenced by the compound content present in the extract. The relationship between antioxidant activity and flavonoid and phenolic concentration was displayed in Table 3.

Table 3. The correlation between phenolic, flavonoid content, and antioxidant activity of ethanol extracts of ginger (*Z. officinale*) rhizome, lemongrass (*C. citratus*) leaves, and mangsian (*P. reticulatus*) fruit in various compositions

	Flavonoid	Phenolic	IC ₅₀
Flavonoid		-0.52043	0.40909
Phenolic	-0.52043		-0.88239
IC ₅₀	0.40909	-0.88239	

The ratio of flavonoid to phenolic content is inversely connected, meaning the higher the concentration of these compounds, the lower the concentration of phenolic. These two categories of substances exhibit various relationships with the IC₅₀ value, which also indicates antioxidant capacity. The phenolic content and the IC₅₀ value are inversely correlated, meaning that the higher the phenolic content, the lower the IC₅₀ value. The lower the IC₅₀ value, the greater the antioxidant ability. Therefore, the ability to act as an antioxidant increases with phenolic concentration. In contrast, a positive association between flavonoid concentration and the IC₅₀ value indicates that the capacity of an antioxidant is lower if the flavonoid content is higher.

Various factors contribute to the antioxidant ability of plant extracts, but one crucial determinant is the compound content present in the extract (Bavisetty & Venkatachalam, 2022; Shekarchian & Soleymani, 2019). This study measures the quantity of flavonoid and phenolic compounds prior to examining the relationship between these two substances and each composition's antioxidant capability. Quercetin was used as a standard flavonoid compound while gallic acid was used as a standard phenolic compound. Both compounds were found abundant in several fruits and medicinal plants (Xu et al., 2019; Kahkeshani et al., 2019). Based on statistically analysis, phenolics have a stronger impact on antioxidant capacity than either of these two substances. The extract's capacity as an antioxidant increases with its phenolic concentration. The flavonoid content, however, exhibits

opposite trend. An et al. (2016) stated that DPPH demonstrated a significant correlation with total phenolic concentration, but a less correlation with total flavonoid concentration. According to the research on *Polygonum minus*, *Z. officinale*, and *Curcuma longa*, plant extracts with higher total phenolic content had increased antioxidant activity, suggesting that phenolic chemicals are important factors of antioxidant activity (Maizura et al., 2011).

CONCLUSION

The antioxidant capacity of single extracts of *Z. officinale* and *P. reticulatus* was strong, while that of *C. citratus* was weak. The composition with three plant samples ZCP 1:0:0, ZCP 0:0:1, and ZCP 1:0:1 was recommended as the ideal composition. Further toxicity research and in vivo testing on that formulation are interesting in order to use it as an antioxidant-rich dietary supplement. Phenolic substances play a major role in antioxidant action.

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