Formulation and Characterization of Instant Powder Combination of Ginger, Bangle, and Lemon Extract as an Antioxidant

Nur Aji¹,²*, Shandra Isasi Sutiswa¹,²
¹Department of Pharmacy, Poltekkes Kemenkes Tasikmalaya, Tasikmalaya, Indonesia
²Center of Excellent Health and Disaster Emergency (CoE HADE) Center Poltekkes Kemenkes Tasikmalaya, Tasikmalaya, Indonesia

*Corresponding author: nuraji090689@gmail.com

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Abstract

Background: Degenerative disease is a decreasing organ function; clinical manifestations can affect the whole body, which is caused by oxidative stress. Ginger, bangle, and lemon have antioxidant properties. The combination of the three is expected to increase antioxidant activity. Objective: This study aimed to determine the potential antioxidant activity of the mixture of the three samples formulated as instant powder. Methods: This research is an experimental laboratory. This study will examine the effect of variations in extract concentration and PEG-40 HCO concentration on instant powder’s characteristics and antioxidant activity. Results: Individually, ginger extract has extreme antioxidant activity (IC₅₀ = 23.57 ± 0.13 µg/mL) and bangle strong (IC₅₀ = 64.89 ± 0.15 µg/mL), while lemon has weak antioxidant activity (IC₅₀ >500 µg/mL). Combining ginger, bangle, and lemon with a simplex axial method obtained the combination of ginger: bangle: lemon with the ratio of 4/6: 1/6: 1/6. Adding a mixture of extracts affects the solubility and antioxidant activity of the extracts. The greater the amount of extract, the lower the solubility, and the antioxidant activity did not increase with addition. The addition of PEG-40 HCO increases the solubility of the extract in the instant powder. Antioxidant activity increased to the “medium” category (121.90 µg/mL) after adding PEG-40 HCO at a concentration of 2.70%. The unfavourable impact of PEG-40 HCO addition on instant powders is the angle of repose, flow time, and compressibility. Conclusion: The ginger, bangle, and lemon can be combined and made into instant powder with potential antioxidant activity in the moderate category.

Keywords: instant powder, ginger, bangle, lemon, antioxidant

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INTRODUCTION
Degenerative disease decreases organ function, which generally affects the elderly. Clinical manifestations of degenerative can affect all organs of the body. Degenerative conditions in Indonesia, such as hypertension, diabetes mellitus, stroke, and chronic kidney failure, increased in 2018 from 2013. The 2018 Basic Health Research results found that stroke prevalence was 7% in 2013 and 10.9% in 2018. The prevalence of chronic kidney disease was 2% in 2013 to 4% in 2018. The prevalence of diabetes mellitus was 1.8% in 2013 to 1.9% in 2018. The prevalence of diabetes mellitus is high in people with higher education and state civil servants (Fridalni, Minropa, and Sapardi, 2019). During the Covid 19 pandemic, people with degenerative diseases have a higher risk of being exposed and tend to experience worse complications from this disease (D. J. E. Sari & Widiharti, 2021).

Degenerative diseases are caused by oxidative stress in the presence of free radicals in biochemical mechanisms that occur in the body. Free radicals are considered dangerous because they become very reactive in trying to get their electron pair (Odinga et al., 2020). Antioxidants can slow down the oxidation process of free radicals, thereby protecting cells from damage caused by unstable molecules known as free radicals (Sadiq, 2023). The mechanism of the body's resistance to oxidative stress is through endogenous antioxidants. If the number of free radicals and reactive species in the body exceeds the ability of endogenous antioxidants, then the body requires the intake of exogenous antioxidants (Martemucci et al., 2022).

Ginger rhizome has antioxidant activity. Ginger oleoresin, as an active substance, can ward off free radicals, such as 6-gingerol and 6-shogaol, which are known to have relatively high antioxidant activity, with a mechanism to stabilize free radicals by complementing the lack of electrons possessed by free radicals and inhibiting the occurrence of chain reactions from the formation of free radicals (Ahmed et al., 2022). Apart from the ginger plant, other plants such as Bangle and Lemon also have antioxidant activity due to the content of flavonoid compounds. In addition, the content of flavonoids in bangle plants also functions as an immunostimulant. In addition, the bangle is rich in curcuminoid compounds, which also have antioxidant potential (Nurkhasanah et al., 2019).

The potential bangle, ginger, and lemon plants, which contain antioxidants, can be used to prevent degenerative diseases (Veurink et al., 2020). In addition, antioxidants are essential to maintain body immunity, especially during a pandemic. A combination of bangle, ginger, and lemon is formulated into an instant powder. The advantages of instant powder formulas are product quality that is more practical and hygienic (Copur et al., 2019). Based on this, researchers are interested in making an instant powder combination of ginger, bangle, and lemon extracts as an antioxidant. This study aims to see the effect of adding a mixture of extracts and PEG-40 HCO on the characteristics of instant powder: solubility, pH, antioxidant activity, angle of repose, flow rate, and powder density. PEG-40 HCO is a solubilizer agent of hydrogenated castor oil derivatives. The hydrogenated castor oil is obtained by hydrogenation of virgin castor oil (Rowe et al., 2009). PEG-40 HCO is known to have the advantages of having good solubility in a reasonably wide polarity range, is non-toxic, and can be used for oral dosage forms (Rachmawati et al., 2017).

MATERIALS AND METHODS
The research was conducted in an experimental laboratory divided into four work stages. The first is the manufacture of extracts and testing of extract parameters. The second stage was optimizing the combination of extracts and instant powder fillers. The third stage is the formulation of ginger and lemon bangle instant powder. The fourth step is to test the characteristics of the extract and the antioxidant activity of the instant powder dosage form.

Instruments and materials
Making the extract involves a macerator and rotary evaporator (InScienPro). In the process of testing the antioxidant activity, total curcuminoids, total flavonoids, and total polyphenols using tools: microanalytical balance (Sartorius), UV-Vis spectrophotometer (Agilent Cary 60), and micropipette (Joanal). Instant powder production involves a dehydrator (Athome), grinder, and other standard glassware (Pyrex). The powder characteristics test involved a pH meter (Dixon Tech), a slide micrometre (Srate), and a centrifuge (Health HC8).

Phytochemical screening using reagents: Mayer, Dragendorff, (DPH), Dragendorff (DPH), sulfuric acid P (Emsure), acetic acid anhydrous (Isolab), FeCl₃ (Sigma), gelatin (Fisher Scientific), gelatin (Fisher Scientific), NaCl (Merck), zinc dust (Emplura), HCl P (Emsure), chloroform (Merck) and ammonia (Emsure). Several standard reagents used in determining extract parameters were gallic acid (Merck), curcumin (Merck), DPPH/1,1-diphenyl-2-picrylhidrazil (Sigma), and Quercetin (Merck). In the manufacturing process, they have used excipients: maltodextrin DE 18-20...
(Ambrosia), Sucralose (Anhui Jinhe), and PEG-40 HCO (Evonik).

The research sample was ginger (Zingiber officinale L.) simplicia obtained from B2P2TOOT. Bangle extract, which was extracted using 96% ethanol (DPH) from the Zingiber montanum (J.Koenig) Link ex A simplicia, plant which had been determined at Herbarium Bandungense ITB with the Voucher code FIPIA-DEP29. Lemon is a local fruit obtained from the C Lemon, which is a local fruit obtained from the city of Tasikmalaya.

**Extraction**

The extract was prepared based on the 2nd Indonesian Herbal Pharmacopoeia, using 96% ethanol. Weighed 1 kg of simplicia was crushed and sieved using mesh number 40. The simplicia powder was then considered, and 995 grams were obtained. The simplicia work was divided into five macerators; ginger simplicia powder was put into the macerator and added with 96% ethanol at a ratio of 1:10. Soak it for the first 6 hours while occasionally stirring and let stand for 18 hours. The solvent replacement was carried out once with the same type and amount, and the treatment was carried out the same way as the first day. All macerate was collected, filtered, and then concentrated in a vacuum rotary evaporator with a speed of 60 RPM, pressure -0.6 mmHg, and a temperature of 60°C. The condensed extract is then calculated in yield using equation 1 (Kemenkes RI, 2017).

\[
\text{Yield (\%)} = \frac{\text{Extract weight (gram)}}{\text{Simplicia weight (gram)}} \times 100\% \quad \text{[1]}
\]

**Test the water content of the extract**

The distillation of toluene determines the water content. First, the toluene is saturated with water; the samples are weighed in 2 grams. The pieces are put into a round bottom flask and added toluene. The toluene distillation device is assembled, making sure the district and burette are clean and dry. The flask was heated for 15 minutes; after the toluene started to boil, the distillation was set at two drops/second. After all the water is distilled, heating is continued for 5 minutes. Allow the receiving tube to cool to room temperature and the water to separate at the bottom of the burette. Moisture content is calculated based on volume percent per sample weight (DepKes, 2000).

**Phytochemical screening**

Phytochemical screening was performed according to Farnsworth (1966) and Hanani (2015) procedures. Phytochemical screening includes compounds: alkaloids, tannins, polyphenols, flavonoids, saponins, triterpenoids, and steroids. The procedure in the following description:

a. For the alkaloids test, the extract is put into a test tube with 28% ammonia, and then 10 mL of chloroform is added. The chloroform phase was separated and acidified with 1 N hydrochloric acid. The acid layer was separated and used for alkaloid testing: with Mayer reagent (Potassium mercuri-iodide solution), the addition of a few drops of Mayer reagent raises a white precipitate, indicating the presence of alkaloids. Dragendorff (Iodobismutat solution): The addition of this reagent raises a brownish-red precipitate, indicating the presence of alkaloids.

b. In the tannin test, the extract is put into a test tube, diluted with 2 mL of water and then divided into two tubes. In the 1st tube, add 2-3 drops of FeCl₃ 1%. The presence of a blue precipitate or blue-black colour indicates the presence of galanin and ellagitaninn, while the green or turquoise colour indicates the presence of condensed tannins. In the 2nd tube, added 1% gelatin (in 10% NaCl) formed a precipitate indicating the presence of tannins.

c. In the polyphenol test, the extract is put into a test tube, diluted with water, and then a few drops of FeCl₃ 1% are added. The presence of a blue precipitate, and blue-black, green and turquoise colours indicate the presence of polyphenolic compounds.

d. In the flavonoid test, the extract was put into a test tube, added 2 mL of 80% ethanol, and then stirred and filtered. Filtrate added 3-4 spatulas of magnesium powder and 0.5 mL of HCl P. The presence of flavonoids is indicated by the production of an orange-to-red hue that can be drawn with amyl alcohol or octyl alcohol.

e. For the saponin test, the extract is put into a test tube with 10 mL of water, then stirred and shaken vigorously vertically for 30 seconds. If the foam is formed with a height of more than 3 cm, which is persistent for 30 minutes, then the sample contains saponin.

f. Testing for steroids and triterpenoids, the extract is put into a test tube, chloroform is added, and then the chloroform phase is separated and evaporated. Three drops of anhydrous acetic acid and one drop of concentrated sulfuric acid were added to the residue (Liebermann-Burchard). The magenta or violet colour formation indicates that the simplicia contains triterpenoid group compounds. The presence of a greenish-blue colour indicates the presence of steroids.
Determination of total curcuminoid levels of bangle extract

Test for total curcuminoids using UV-Vis spectrophotometry (Kemenkes RI, 2017). Preparation of the test solution begins by carefully weighing approximately 100 mg of the extract, placing it in an Erlenmeyer flask, and adding 10 mL of ethanol P, sonicating until the extract is dissolved. Filter into a 10 mL volumetric flask, rinse the filter paper with ethanol P, and add ethanol P up to volume. The sample solution was diluted ten times by taking 0.1 mL of input into a 10 mL volumetric flask and adding ethanol P up to the mark. A reference solution was prepared by carefully weighing approximately 10 mg of curcumin, putting it into a 10 mL volumetric flask, and adding ethanol P up to the mark. Make a series of dilutions for the reference solution with levels 1, 2, 3, 4, 5, and 6 µg/mL, respectively. Blank solution using Ethanol P. Measurements were made by pipetting 3 mL of the test solution separately, each series of the Reference solution and the Blank solution into the appropriate containers, measuring the absorbance at the maximum absorption wavelength of approximately 420 nm. Create a calibration curve to determine the regression equation. The sample concentration is calculated in weight percent (% w/w) with equation 2 where k is the curcuminoid concentration in µg/mL, f is the dilution factor, v is the sample volume before dilution (mL), and w is the sample weight.

\[
\text{Curcuminoid (\%) = } \frac{k \times v}{w} \times 100\% \quad \quad \quad \quad \quad \quad [2]
\]

Determination of total polyphenol levels

The reference solution was diluted ten times for a 100 µg/mL concentration. The reference solutions were made in serial dilutions with successive concentrations of 1, 2, 3, 4, and 5 µg/mL, as seen in Table 1.

A total of 1 mL of the test solution was taken, and each series of reference solutions was put into the appropriate container; at each concentration, 0.5 mL of F-C solution was added. The mixture was allowed to stand for 8 minutes, then 0.5 mL of saturated Na2CO3 was added and incubated for 1 hour at room temperature, after which 10 mL of water was added. The wavelength absorption of each solution was measured at a wavelength of 740 nm. Blank measurements were carried out the same way, without adding the test solution. A calibration curve was created, and the gallic acid equivalent (GAE) sample concentration was calculated (Aji, Kumala, et al., 2023; Kemenkes RI, 2017).

Determination of total flavonoid levels

Weighed 0.1 gram of sample and put it into an Erlenmeyer, then 10 mL of ethanol P sonicated was added until the extract dissolved. Filter into a 10 mL volumetric flask, rinse the filter paper with ethanol P, and add up to the mark. The reference solution was prepared by carefully weighing 10 mg of quercetin, putting it into a 10 mL volumetric flask, dissolving it, and adding ethanol P up to the mark. Make serial dilutions of 20, 30, 40, 50, and 60 µg/mL solutions. The assay started by pipetting 0.5 mL of the test solution separately and each series of reference solutions into the appropriate container, adding 1.5 mL of ethanol P, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M sodium acetate, and 2.8 water (Table 2). Shake the solution for 30 minutes at room temperature. Measure the absorbance at \(\lambda_{\text{max}}\). Create a calibration curve and calculate the levels in the presence of quercetin equivalent (QE) flavonoids (Islam et al., 2022; Kemenkes RI, 2017).

Table 1. Series of dilution tests for total polyphenol content by gallic acid equivalence method

<table>
<thead>
<tr>
<th>No.</th>
<th>Gallic Ac. (mL)</th>
<th>F-C (mL)</th>
<th>Na₂CO₃ (mL)</th>
<th>Water (mL)</th>
<th>Final Volumes (mL)</th>
<th>Gallic Acid Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.50</td>
<td>0.50</td>
<td>9.00</td>
<td>10.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
<td>0.50</td>
<td>0.50</td>
<td>8.90</td>
<td>10.00</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>0.20</td>
<td>0.50</td>
<td>0.50</td>
<td>8.80</td>
<td>10.00</td>
<td>2.00</td>
</tr>
<tr>
<td>4</td>
<td>0.30</td>
<td>0.50</td>
<td>0.50</td>
<td>8.70</td>
<td>10.00</td>
<td>3.00</td>
</tr>
<tr>
<td>5</td>
<td>0.40</td>
<td>0.50</td>
<td>0.50</td>
<td>8.60</td>
<td>10.00</td>
<td>4.00</td>
</tr>
<tr>
<td>6</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>8.50</td>
<td>10.00</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Table 2. Determination of total flavonoids by quercetin equivalence method

<table>
<thead>
<tr>
<th>Quercetin (µg/mL)</th>
<th>Vol. Quercetin (mL)</th>
<th>AlCl₃ (mL)</th>
<th>Na-Acetat (mL)</th>
<th>Ethanol (mL)</th>
<th>Water (mL)</th>
<th>Quercetin Final Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.00</td>
<td>0.50</td>
<td>0.10</td>
<td>0.10</td>
<td>1.50</td>
<td>2.80</td>
<td>2.00</td>
</tr>
<tr>
<td>30.00</td>
<td>0.50</td>
<td>0.10</td>
<td>0.10</td>
<td>1.50</td>
<td>2.80</td>
<td>3.00</td>
</tr>
<tr>
<td>40.00</td>
<td>0.50</td>
<td>0.10</td>
<td>0.10</td>
<td>1.50</td>
<td>2.80</td>
<td>4.00</td>
</tr>
<tr>
<td>50.00</td>
<td>0.50</td>
<td>0.10</td>
<td>0.10</td>
<td>1.50</td>
<td>2.80</td>
<td>5.00</td>
</tr>
<tr>
<td>60.00</td>
<td>0.50</td>
<td>0.10</td>
<td>0.10</td>
<td>1.50</td>
<td>2.80</td>
<td>6.00</td>
</tr>
</tbody>
</table>
Table 3. Dilution series and addition of DPPH test reagents

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Methanol (mL)</th>
<th>Sample (mL)</th>
<th>DPPH (mL)</th>
<th>Sample Concentration (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.00</td>
<td>0.00</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>A1</td>
<td>3.75</td>
<td>0.25</td>
<td>1.00</td>
<td>5.00</td>
</tr>
<tr>
<td>A2</td>
<td>3.50</td>
<td>0.50</td>
<td>1.00</td>
<td>10.00</td>
</tr>
<tr>
<td>A3</td>
<td>3.00</td>
<td>1.00</td>
<td>1.00</td>
<td>20.00</td>
</tr>
<tr>
<td>A4</td>
<td>2.00</td>
<td>2.00</td>
<td>1.00</td>
<td>40.00</td>
</tr>
<tr>
<td>A5</td>
<td>0.00</td>
<td>4.00</td>
<td>1.00</td>
<td>80.00</td>
</tr>
</tbody>
</table>

Antioxidant activity assay of ginger extract, bangle, and lemon juice

Sample stock solutions and 1,1-difenil-2-pikrilhidrazil (DPPH) were prepared at a concentration of 100 μg/mL, then the samples and DPPH were reacted as shown in Table 3, with a total volume of 5 mL to obtain sample concentrations of 5, 10, 20, 40, and 80 μg/mL. The solution concentration series were incubated at 37°C for 30 minutes and protected from light. After that, the absorbance at a wavelength of 517 nm was calculated using a UV-Vis spectrophotometer (Agilent Carry 60) and the percent inhibition (Pi) using equation 3. The value of Ab is the absorbance of the control, while As is the absorbance of the sample. The results of the calculation are entered in a linear regression equation with the extract concentration (μg/mL) as the abscissa (x-axis) and the % inhibition activity value as the coordinate (y-axis). The IC50 value is calculated by the equation y = ax + b (Aji, Puspitasari, et al., 2023; Sirivibulkovit et al., 2018). The IC50 category can be seen in Table 4.

\[ Pi = \frac{Ab - As}{Ab} \times 100\% \] .................[3]

Table 4. Category IC50 of antioxidant activity
(Zamzani & Triadistri, 2021)

<table>
<thead>
<tr>
<th>Antioxidant Intensity</th>
<th>Score IC50 (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Strong</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Strong</td>
<td>50-100</td>
</tr>
<tr>
<td>Currently</td>
<td>100-250</td>
</tr>
<tr>
<td>Weak</td>
<td>250-500</td>
</tr>
</tbody>
</table>

Antioxidant assay combination of ginger extract, bangle, and lemon juice

The simple axial approach is used to determine combinations involving three samples simultaneously. This approach estimates the optimal mix of extracts, minimizing the amount used. (Cavalcanti et al., 2021). The combinations follow Figure 1 and Table 5. Combination 1, ginger, bangle and lemon extracts were 1/3: 1/3: 1/3, combination 2 is 4/6: 1/6: 1/6, combination 3 is 1/6: 1/6: 4/6, and combination 4 is 1/6: 4/6: 1/6 (Cornell, 2011).

Figure 1. A simple axial diagram of the ginger-bangle-lemon combination (Cornell, 2011)

Table 5. Combinations of ginger extract: bangle: lemon

<table>
<thead>
<tr>
<th>Combination</th>
<th>X1 (Ginger)</th>
<th>X2 (Bangle)</th>
<th>X3 (Lemon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/3</td>
<td>1/3</td>
<td>1/3</td>
</tr>
<tr>
<td>2</td>
<td>4/6</td>
<td>1/6</td>
<td>1/6</td>
</tr>
<tr>
<td>3</td>
<td>1/6</td>
<td>1/6</td>
<td>4/6</td>
</tr>
<tr>
<td>4</td>
<td>1/6</td>
<td>4/6</td>
<td>1/6</td>
</tr>
</tbody>
</table>

All formula combinations were made into 100 μg/mL stock concentrations and then made into five concentration variations and one blank. Each concentration was added with 100 μg/mL DPPH (Table 3); then, the absorbance was measured. The next step was calculating the percent inhibition and making a linear regression curve, which sought the IC50 value. The sample stock solution was diluted into five graded concentration variations. Add 1.0 mL of 150 mg/L DPPH solution until the total volume of the solution is 5.0 mL (Julizan, 2019).

Measure the absorbance of the solution using a spectrophotometer at a wavelength of 517 nm, calculate the inhibition ability of each sample concentration as a percent inhibition (equation 3), and Plot the sample's concentration value and % inhibition on the standard curve. The concentration of 50% inhibition (IC50) is determined using the linear regression.
Instant powder formulation with the added combination of extracts

The formulation was based on a combination of extracts with the highest antioxidant activity. The selected extract combinations were then made into instant powder with increased concentrations of 100, 200, 300, 400, and 500 times their IC₅₀ values. The instant powder is formulated with the addition of fillers maltodextrin dextrose equivalent (DE) 18-20 and sucralose as a sweetener. Sucrose was used at a concentration of 0.24%. Maltodextrin is chosen with DE 18-20, which is very soluble in water. The formula uses maltodextrin as a filler with an amount of 1-99% (Rowe et al., 2009). The instant powder formula can be seen in Table 8. After being formulated, the instant powder was tested for antioxidant activity, organoleptic observation, solubility, sedimentation, and clarity tests. Antioxidant activity test using a sample weight of 100 mg.

Instant powder formulation with the addition of PEG-40 HCO

PEG-40 Hydrogenated Castor Oil or PEG-40 HCO is a surfactant that can help dissolve instant powder in water (Kadian & Nanda, 2022). The addition of PEG-40 HCO is expected to increase antioxidant activity by increasing the solubility of instant powder when dissolved in water. The instant powder formula with the successive addition of PEG-40 HCO from 0, 1, 3, 9, and 27% can be seen in Table 9. After the formulation, the antioxidant activity was tested with a sample size of 100 mg. Powder characteristic test includes parameters: organoleptic, dissolving time, clarity, sedimentation, angle of repose, flow time, bulk density, incompressible density, and Hausner factor. In addition to the characteristics of the formula with the best antioxidant activity, a proximate test was also carried out.

Instant powder characteristics test

Instant powder organoleptic was carried out macroscopically and microscopically. Macroscopic observations include the form of aroma, colour, and taste. The microscopic observation of powder particle shape. Microscopic observation using a binocular microscope with a slide micrometre. The instant powder is put on the micrometre slide, dripped with liquid paraffin, and covered using a cover glass.

The solubility test was performed by dissolving instant powder in water with a ratio of 1:10 up to 1:100 at 10 mL volume intervals. They stirred the solution using a magnetic stirrer at a speed of 2400 rpm for 5 minutes. Percent transmittance (%T) is used to measure the clarity of a solution or dispersion system quantitatively. A high %T value means that the particle size is getting smaller, and the level of transparency is increased (Abdassah, 2017). The turbidity of the solution was measured using UV-Vis photometer spectroscopy at a wavelength of 800 nm, and the %T value was calculated. The sedimentation test carried out the solubility test results by placing the sample in a centrifugation tube and rotating it at 4000 rpm for 5 minutes. The centrifugation results were then seen for the presence or absence of sediment.

The dissolution time test was carried out by weighing 1 gram of instant powder in a 100 mL glass, and then the dissolving time of the powder in water was recorded. The time needed to dissolve is less than 5 minutes (Schorsch et al., 2001).

The angle of repose is a fixed angle between the heap of conical particles and the horizontal plane when a certain amount of powder is poured into the measuring device. A good angle of silence between 25- 40°. The angle of repose is determined by the equation Tan α = h/r, where α is the angle of repose, h is the height of the cone, and r is the radius of the cone (Husni et al., 2020).

The flow time is determined by weighing 25 grams of powder poured into the measuring funnel. The funnel lid is opened slowly; the granules are allowed to flow out. Time was recorded with a stopwatch until all the granules flowed out. A good flow time is ≤10 grams/second or 100 grams ≤ 10 seconds (Hudha & Widyaningsih, 2015).

The pH value is determined by weighing 1 gram of instant powder dissolved in 100 mL of distilled water. Take measurements using a calibrated pH meter in the test range using a standard buffer.

The bulk density (ρ) test was carried out by weighing 30 grams of powder (Wo), then putting it into a 100 mL measuring cup and observing its volume (Vo). The formula calculates the bulk ρ value: Wo/Vo. The compress ρ test is carried out by weighing 30 grams of powder (Wo) input into a 100 mL measuring degree and measuring the volume (Vt). Then, place it on the tap density tester by tapping 1,250 times and recording the volume (Vt1). If the difference between Vt and Vt1 is not more than 2 mL, then Vt is used. The Wo/Vt formula calculates the compress ρ value. The incompressible ρ value is affected by the particle size (Abdullah & Imtihani, 2022). The Hausner factor is a method of determining the flow properties of powders by measuring ρ bulk and ρ compressed. A ratio of <1.25 indicates good characteristics, and >1.50 means poor characteristics (Kusumo & Mita, 2018). In addition to the Hausner ratio, the percent (%) compressibility value.
is also specified. The Hausner factor is calculated using equation 4, and % compressibility using equation 5.

Hausner Factor = $\frac{\rho_{\text{Compress}}}{\rho_{\text{Bulk}}}$ ...................................[4]

% Compressibility = $\frac{\rho_{\text{Compress}} - \rho_{\text{Bulk}}}{\rho_{\text{Compress}}}$ ....................[5]

**Data analysis**

Descriptive analysis was done for the parameter data of extract and powder characteristics. In comparison, regression analysis determines total curcuminoid levels, total polyphenols, total flavonoids, and antioxidant activity.

**RESULTS AND DISCUSSION**

**Extraction results and extract parameters test**

Bangle is an extract obtained by the maceration method using 96% methanol. Bangle extract was brought based on previous research by Aji et al. (2023). Ginger extract is made using the maceration method with 96% ethanol solvent. The result of ginger extraction yielded 8.74%. In contrast, in lemon fruit juice, from 500 grams of fresh fruit, 120 mL of liquid is obtained. Water level testing was done on ginger and bangle extracts. The water content obtained was as much as 5.64±1.63% for ginger extract and 9.09±0.61% for bangle extract. The water content of both extracts meets the Indonesian Herbal Pharmacopoeia requirements (Kemenkes RI, 2017).

Phytochemical screening aims to identify the content of secondary metabolite compounds of a natural material. The results of phytochemical screening can be seen in Table 6. Plants are an essential source of bioactive compounds for developing new therapeutic agents. Some of them have antioxidant activity. Based on Table 6, concentrated extracts of ginger and bangle were identified as having the same group of compounds: alkaloids, polyphenols, flavonoids, and triterpenoids. While lemon identified only polyphenols. Several secondary metabolite compounds, such as polyphenols and flavonoids, are known to have antioxidant activity (Nhu-Trang et al., 2023).

Total curcuminoid level testing was done on the bangle, associated with the yellow pigment content caused by complex curcuminoid content such as cassumunin A, B, and C (Kabkrathok et al., 2022). The total curcuminoid level testing result from the bangle is 1.82±0.01%. This value is much lower than the previous research conducted by Aji et al. (2023), which amounted to 3.84%. The low level of total curcuminoid can be caused by several factors in the extract storage process, such as temperature, water content, and pH. Curcuminoids will be stable at acidic pH (<7) in aqueous solution and very unstable at neutral and alkali pH (>7). In contrast, high temperature is a catalyst for accelerating curcuminoid degradation (Kharat et al., 2017).

**Table 6. Results of phytochemical screening**

<table>
<thead>
<tr>
<th>No.</th>
<th>Group of Compounds</th>
<th>Ext.</th>
<th>Ext.</th>
<th>Lemon Juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Polyphenol</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Tannin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Triterpenoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) present, (-) absent

Total polyphenol (Figure 2a) and total flavonoid (Figure 2b) levels were tested on all three samples. The test results can be seen in Figures 2a and 2b. The highest level of phenols is found in the ginger extract, followed by bangle and lemon, with the lowest concentration. In this study, the total polyphenol content of ginger was 1.22 mgGAE/g compared to previous research by Ghafoor et al. (2020), where the total polyphenol content was much higher at 9.32 mgGAE/g. The low polyphenol content may occur due to the simplified heating-drying process. In previous studies, the drying method of simplicia used freeze-dried so that the oxidation of polyphenolic compounds could be minimized with low temperatures. Similarly, the total polyphenol content in this study obtained 0.64 mgGAE/g in previous research by (2022), which amounted to 3.81 mgGAE/g. Both used the same extraction method and solvent. The main content of ginger rhizome is oleoresin in the form of gingerol and shogaol derivatives (Van et al., 2023). The gingerol and shogaol compounds (Figures 3a and 3b) are polyphenolic compounds that can react with the F-C reagent and be read in the test (Nikolaeva et al., 2022). In the bangle, the main compound is curcuminoid (Figure 3c), which has a polyphenol functional group and can react with the F-C reagent. The lowest content is lemon juice; unlike ginger and bangle extract, lemon is not concentrated, so it has the lowest range.
Different from the total polyphenol content test in the total flavonoid test, the bangle has the highest flavonoid content compared to the other two samples. For total flavonoid levels in this study, it was 2.01 mgQE/g, based on previous research by (2022), with levels of 1.69 mgQE/g. In this study, flavonoid levels were slightly higher than in the previous study. This study and the previous one were ginger rhizomes sourced from the same harvest site. One of the reasons for the difference is the different harvest times. As for bangle in, this study has a level of 17.04 mgQE/g, while in the previous research it was 21.93 mgQE/g. This and previous studies used the same extraction method and solvent but different sources. Based on this, the drying method, harvest time and harvest location significantly affect the levels of total polyphenol content and total flavonoids. Based on the research of Hassan et al. (2019) besides curcuminoid bangle is known to contain five flavonoid derivatives, namely kaempferol 3-O-rhamnopyranoside, kaempferol 3-O-(3"-O-acetyl) rhamnopyranoside, kaempferol 3-O-methyl ether, kaempferol 3-O-(4"-O-acetyl) rhamnopyranoside, and kaempferol 3-O-(3,4"-di-O-acetyl) rhamnopyranoside.

**Antioxidant test results of ginger extract, bangle, and lemon juice individually and in combination**

The results of the antioxidant activity test of the three samples can be seen in Table 7. The antioxidant activity is correlated with the total polyphenol level; the higher the total polyphenol level in the extract, the higher the antioxidant activity (Muflihah et al., 2021). The IC\(_{50}\) value is the concentration of the sample solution needed to inhibit 50% of DPPH free radicals. The lower the IC\(_{50}\) value, the higher the activity (Ramadhan et al., 2022). Ginger extract has very strong antioxidant activity (<50 μg/mL), the bangle has strong activity (50-100 μg/mL), and lemon has weak activity (> 500 μg/mL) (Zamzani & Triadisti, 2021).
Table 7. Antioxidant activity of ginger, bangle, and lemon

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC50 (μg/mL)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ext. EtOH 96% Ginger</td>
<td>23.57</td>
<td>0.13</td>
</tr>
<tr>
<td>Ext. EtOH 96% Bangle</td>
<td>64.89</td>
<td>0.15</td>
</tr>
<tr>
<td>Lemon Juice</td>
<td>4,268.02</td>
<td>0.11</td>
</tr>
</tbody>
</table>

SD : Standard deviation

Based on the results (Figure 4), the antioxidant activity of combination number two with the composition of ginger (4/6), bangle (1/6): and lemon (1/6) is the composition that has the highest activity with the category of "medium" potential. The combination is done using a simple axial. Although lemon juice has weak antioxidant activity, it contains organic acids that can stabilize curcuminoids (Asencio et al., 2018; Zheng et al., 2017). Curcuminoid compounds are stable in aqueous solutions at acidic pH (<7) (Manju & Sreenivasan, 2011). The content of organic acids such as citric acid and malic acid in the lemon will create acidity in the extract combination (Sánchez-Bravo et al., 2022). The combination of extracts has lower antioxidant activity than single extracts (ginger and bangle). The decrease may occur due to dilution from the effect of mixing extracts that have intense antioxidant activity with weak ones (Harun & Rahmawati, 2022). In addition to antioxidant activity, the working mechanism of the characteristic components in the extract can be used as a target in combination design. For example, 6-gingerol regulates lipogenesis, fatty acid oxidation, mitochondrial dysfunction, and oxidative stress (Cerdá et al., 2022). Curcumin affects the treatment of diabetes by improving β-cell function, preventing β-cell death, and reducing insulin resistance (Quispe et al., 2022).

**Instant powder formulation results with the addition of extract combinations**

Based on the optimization results, combination number two was selected to proceed to the formulation stage. The IC50 value of combination extract number two is 121.90 μg/mL or 0.0122%. At this stage, the effect of adding extracts on instant powder's characteristics and antioxidant activity will be seen. The extracts were increased to 100, 200, 300, 400, and 500 times from the IC50 value. The formula can be seen in Table 8.

The result of organoleptic observation (Figure 5) macroscopically shows that the dosage form is pale yellow to yellow. The yellow colour's intensity increases as the added extract's concentration increases. Similarly, the aroma will increase with the addition of extracts. All instant powders have a powder form with a smooth texture. The instant powder has a sweet, sour, and spicy taste. The sour and spicy flavour will increase with the addition of extract. The results of microscopic (Figure 6) observation of all formulas (F1 to F5) have a crystal shape with a heterogeneous structure and size.

Table 8. Formulation of instant powder with the addition of combination extract number 2

<table>
<thead>
<tr>
<th>No.</th>
<th>Materials</th>
<th>Concentration % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>1</td>
<td>Combination of</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>Extracts 2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sucralose</td>
<td>0.24</td>
</tr>
<tr>
<td>3</td>
<td>Maltodextrin</td>
<td>98.54</td>
</tr>
<tr>
<td></td>
<td>DE 18-20</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 5. The appearance of instant powder

Figure 6. Microscopic appearance of instant powder on a micrometre slide (1 Division = 0.01 mm)
The effect of adding extract combination on the antioxidant activity can be seen in Figure 7. The instant powder antioxidant activity from formula F1 to formula F3 shows an increase in antioxidant activity marked by a decrease in the IC$_{50}$ value. However, in formulas F3, F4, and F5, the increase in concentration is not directly proportional to the increase in antioxidant activity, which is shown by a graph that slopes toward the average. The saturation of the solution causes the phenomenon; the higher the concentration of the extract, the more precipitates are formed. In line with the theory, it is supported by the results of testing the clarity of the instant powder solution shown in Figure 8. The results of the clarity test show that the higher the extract concentration, the lower the percentage of transmittance (%T) will be. The decreasing value of %T shows the increasing value of turbidity. In addition, the result of qualitative sedimentation testing in Figure 9 shows that the higher the extract is added, the more sediment is formed. One of the efforts to increase the solubility of the extract in the instant powder is by adding surfactants such as PEG-40 HCO, a polyoxyethylene derivative of castor oil with an HLB value of 15.

**Instant powder formulation with PEG-40 HCO addition**

Based on optimization, adding extracts affects antioxidant activity and powder instant characteristics. Instant powder formula F5 produces the highest activity but low solubility. The decrease in solubility is caused by the high concentration of the extract so that saturation occurs. The solubility of formula F5 can be improved by adding surfactants, one of which is PEG-40 HCO. The addition of surfactant is expected to increase instant powder's solubility and antioxidant activity. The instant powder formula with the addition of PEG-40 HCO can be seen in Table 9.

**Table 9.** Instant powder formula with the addition of PEG-40 HCO

<table>
<thead>
<tr>
<th>No.</th>
<th>Materials</th>
<th>Concentration % (w/w)</th>
<th>F5a</th>
<th>F5b</th>
<th>F5c</th>
<th>F5d</th>
<th>F5e</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extract Combination 2</td>
<td>6.10</td>
<td>6.10</td>
<td>6.10</td>
<td>6.10</td>
<td>6.10</td>
<td>6.10</td>
</tr>
<tr>
<td>2</td>
<td>PEG 40 HCO</td>
<td>0.00</td>
<td>0.10</td>
<td>0.30</td>
<td>0.90</td>
<td>2.70</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sucralose</td>
<td>0.24</td>
<td>0.24</td>
<td>0.24</td>
<td>0.24</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Maltodextrin DE 18-20</td>
<td>93.66</td>
<td>93.56</td>
<td>93.36</td>
<td>92.76</td>
<td>90.96</td>
<td></td>
</tr>
</tbody>
</table>
The results of organoleptic observation (Figure 10) of the five formulas do not have any characteristic differences. The five formulas have a yellow colour, a typical bangle aroma, a sour, spicy, sweet taste, a powder form, and a smooth texture. Likewise, from the microscopic observation (Figure 11), the powder from formula F5a to formula F5e did not experience any change in morphology. Microscopically, instant powder has a crystal shape.

The bias of the effect of adding excipients on antioxidant activity, all excipients were tested for antioxidant activity. The results were that PEG-40 HCO, maltodextrin DE 18-20, and sucralose did not have antioxidant activity. Clarity test results (Figure 13b) show that the higher the addition of PEG-40 HCO, the higher the %T, which means the more transparent the solution. The results of the sedimentation test can be seen in Figure 14. The higher the addition of PEG-40 HCO, the less sediment formed, and the intensity of the yellow colour increases. The solubility test results show that all formulas (F5a to F5e) dissolve in less than 5 minutes. The test results prove that adding PEG-40 HCO increases the solubility of the extract from the instant powder, which impacts the increase in antioxidant activity (Figure 13a).

One of the reasons for using PEG-40 HCO is the ability to solubilize well ginger and bangle extracts containing water-insoluble oleoresin and curcumin, which PEG-40 HCO can enhance with a relatively small concentration of 2.70%. The phenomenon of increased solubility by surfactants is due to micellar solubilization. An important property of micelles that has a special meaning in pharmaceuticals is their ability to increase solubility, the spontaneous dissolution of a substance through a reversible interaction with surfactant micelles in water to form a thermodynamically stable isotropic solution (A. K. Sari & Nurihardiyanti, 2023). The extract is a multi-component that has hydrophilic to hydrophobic compounds. There are several possible solubility loci for active substances in micelles, as shown in Figure 12.
Hydrophilic compounds can be adsorbed on the surface of micelles (1); drugs with intermediate solubility should be placed in the middle position in micelles, such as between the hydrophilic head groups of micelles (2) and in the palisade layer between the hydrophilic group and the first few carbon atoms of the hydrophobic group, which is the outer core (3), and hydrophobic drugs that are not soluble at all can be placed in the micelle core (4) (Rangel-Yagui et al., 2005).

The angle of rest is one of the parameters of powder flow properties. Based on Figure 15, the higher the addition of PEG-40 HCO, the higher the angle of repose. An excellent quiet angle is 25-40°. So, in the formula F5c-F5e, the powder has a wrong angle of repose. The shape and size of the particles influence the angle of repose. The finer the particle size of a mass or material, the more difficult it is to flow and form an angle with a high slope. The larger the angle produced, the worse the powder flow quality (Mehrabi et al., 2023). According to the theory, the addition of PEG-40 HCO (Figure 16) affects the flow rate; the higher the PEG-40 HCO is added, the more the powder flow rate decreases. The five formulas do not meet the requirements where the good powder has a flow speed of more than 10 grams/second (Sarabandi et al., 2019). In addition, PEG-40 HCO is a liquid substance that cannot be dried at the temperature of drying powder it can increase the cohesion and adhesion of particles. This is likely to be...
one of the factors in the deterioration of the powder flow.

The formulas of F5a-F5e have a pH in the range of 4.16-4.20 (Figure 17); in this value degree, the formulas have an acidic nature due to the addition of lemon juice. The acidic pH value of the instant powder aims to increase the stability of the curcuminoids in the powder instant. Curcuminoids will decompose at neutral to alkaline pH (Zheng & McClements, 2020). Curcumin is more stable in oily or acidic pH environments. (Martínez-Guerra et al., 2019; Zheng et al., 2017).

The results of the powder density test can be seen in Figure 18. The Hausner ratio (Figure 18a) of all formulas (F5a to F5e) shows good characteristics (<1.25). The compressibility values (Figure 18b) of F5a, F5b, and F5c are in a poor category (26 to 31%), while the formulas F5d and F5e are in a reasonably good category (20 to 25%) (Kusumo & Mita, 2018).

The proximate test was carried out on the formula with the highest antioxidant activity, the F5e formula. They were tested carrying out in the Laboratory UNPAD Central. The test results can be seen in Table 10.

Table 10. Proximate value of instant powder with the addition of PEG-40 HCO

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameters</th>
<th>Concentration (%)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water content</td>
<td>5.58</td>
<td>Gravimetry</td>
</tr>
<tr>
<td>2</td>
<td>Ash Content</td>
<td>0.10</td>
<td>Gravimetry</td>
</tr>
<tr>
<td>3</td>
<td>Fat level</td>
<td>5.90</td>
<td>Soxhlet</td>
</tr>
<tr>
<td>4</td>
<td>Protein Content</td>
<td>0.21</td>
<td>Kjeldahl</td>
</tr>
<tr>
<td>5</td>
<td>Carbohydrate Content</td>
<td>88.21</td>
<td>By Difference</td>
</tr>
<tr>
<td>6</td>
<td>Total Sugar Content</td>
<td>18.09</td>
<td>Anthrone</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Ginger and bangle have robust (IC$_{50}$ = 23.57 ± 0.13 µg/mL) and strong (IC$_{50}$ = 64.89 ± 0.15 µg/mL) antioxidant activities, while lemon has weak activity (IC$_{50}$ > 500 µg/mL). The combination of bangle ginger and lemon using the simple Axial method obtained combination No.2 with the composition ginger (4/6): bangle (1/6): lemon (1/6). This combination has moderate activity (121.90 µg/mL). Combination No. 2 extracts can be formulated in instant powder. The formula with the highest antioxidant activity is the F5 formula, with a concentration increase of 500 times the IC$_{50}$ value. Adding PEG-40 HCO to the F5 formula aims to improve the solubility of the extract in instant powder.

Results The addition of PEG-40 HCO affected the solubility of the extract and antioxidant activity. After adding PEG-40 HCO, the solubility and antioxidant activity increased. However, with the addition of PEG-40 HCO, some of the characteristics of the instant powder experienced a decrease in quality, such as angle of repose, flow time, and compressibility.

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**AUTHOR CONTRIBUTIONS**


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**CONFLICT OF INTEREST**

The authors declared no conflict of interest.

**REFERENCES**


