JURNAL TEKNOSAINS

VOLUME 13 No. 2, 22 Juni 2024 Halaman 162-174

KINETIC OPTIMIZATION OF ANGKAK – RED GINGER EXTRACTION AND ITS IMPACT ON ANTIOXIDANT ACTIVITY

OPTIMASI KINETIK DALAM EKSTRAKSI ANGKAK – JAHE MERAH DAN DAMPAKNYA TERHADAP AKTIVITAS ANTIOKSIDAN

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Submitted: 2023-07-16; Revised: 2023-11-10; Accepted:2023-11-13

ABSTRAK

Campuran angkak dan jahe merah merupakan ramuan herbal masyarakat Tionghoa yang berpotensi sebagai antioksidan. Agar lebih efisien, kombinasi herbal tersebut dapat diekstraksi dengan metode maserasi panas kinetik dengan melakukan optimasi pada kecepatan pengadukan. Beberapa penelitian sebelumnya menunjukkan bahwa penggunaan kecepatan pengadukan pada 400, 600, dan 800 rpm memberikan ic_{50} dan rendemen terbaik. Berdasarkan permasalahan tersebut, penelitian ini bertujuan untuk mengetahui pengaruh peningkatan kecepatan pengadukan terhadap IC_{50} dan % rendemen pada ekstraksi kombinasi jahe merah dan angkak, serta membuktikan manfaat kombinasi herbal tersebut melalui nilai IC_{50}. Metode yang digunakan yaitu: mengekstraksi campuran angkak dan jahe merah pada variasi kinetik 400, 600, dan 800 rpm menggunakan magnetic hotplate stirrer pada suhu 60ºC selama 2 jam; penentuan % rendemen; penentuan profil kromatogram dengan KLT; penentuan aktivitas antioksidan menggunakan DPPH; serta analisis data persentase rendemen dan IC_{50} menggunakan SPSS. Analisis dengan SPSS menunjukkan bahwa kecepatan pengadukan berpengaruh signifikan terhadap % rendemen, dimana peningkatan kecepatan pengadukan di atas 400 rpm mengakibatkan penurunan terhadap % rendemen. Meskipun demikian, kecepatan pengadukan tidak berpengaruh terhadap IC_{50}. Profil kromatogram KLT menunjukkan adanya senyawa 6-gingerol pada jahe merah serta senyawa pigmen merah dan kuning pada angkak yang memiliki potensi sebagai antioksidan. Hasil optimasi pada penelitian ini memperoleh % rendemen dan IC_{50} optimal pada 400 rpm dengan nilai rata-rata berturut-turut yaitu 15,933±3,4771% dan 103,76±10,032 ppm, serta kombinasi bahan angkak dan jahe merah dapat bekerja secara sinergis untuk meningkatkan rendemen maupun antioksidan.

Kata kunci: *Maserasi, Panas-Kinetik, Jahe, Antioksidan, Rendemen*

ABSTRACT

A mixture of angkak and red ginger is a Chinese herbal concoction with potential as an antioxidant. This herbal combination can be extracted using the kinetic hot maceration method by optimizing the stirring speed to be more efficient. Previous studies have shown that using 400, 600, and 800 rpm stirring speeds provides the best IC_{50} and yield. Based on these problems, this research aims to determine the effect of increasing stirring speed on IC_{50} and % yield in the extraction of angkak and red ginger, as well as prove the benefits of this herbal combination through the IC50 value. The method used is extracting a mixture of angkak and red ginger at kinetic variations of 400, 600, and 800 rpm using a magnetic hotplate stirrer at a temperature of 60ºC for 2 hours; determination of % yield; determining the chromatogram profile using TLC; determination of antioxidant activity using DPPH;

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as well as data analysis of percentage yield and IC_{50} using SPSS. Analysis using SPSS shows that the stirring speed significantly affects the % yield, where increasing the moving speed above 400 rpm results in a decrease in the % yield. However, the moving speed does not affect the IC_{50}. The TLC chromatogram profile shows the presence of 6-gingerol compounds in angkak and red and yellow pigment compounds in red ginger, which have potential as antioxidants. The optimization results in this study obtained optimal % yield and IC_{50} at 400 rpm with average values, respectively, namely 15.933 ± 3.4771% and 103.76 ± 10.032 ppm, and the combination of angkak and red ginger ingredients can work synergistically to increase yield and antioxidant.

Keywords: *Maceration, Heat-Kinetic, Red Ginger, Antioxidant, Yield*

INTRODUCTION

Chinese culture is renowned for its herbal remedies, with one notable example being a mixture of angkak and ginger. This concoction is cherished for its reputed ability to rejuvenate the body and remedy various ailments. Angkak is a nutraceutical made from fermented rice with *Monascus purpureus* yeast and has long been used by Chinese residents as food coloring, seasoning, and traditional medicine. Ginger is a well-known plant valued for its rhizomes, used as food, seasoning, and traditional medicine. One of the scientific reasons that can form the basis for the combination of the two herbs is the content of their compounds, which have been shown to have antioxidant properties, namely *gingerol* and *shogaol* in ginger, as well as the pigment in angkak (Basuny & Abdel-Raheam, 2020; Ooi *et al.*, 2021).

The consumption of this herb found in the community still uses the traditional method, namely by boiling. As a result, it is less practical to carry because it is a large volume and is difficult to formulate. Hence, it needs to be made into an extract with a smaller volume with effectiveness equivalent to Simplicia to facilitate the adjustment of dosage and formulation. Maceration, one of the most employed extraction techniques, has been historically applied to angkak and Ginger owing to its simplicity in equipment setup, ease of implementation, and cost-effectiveness (Munadi, 2018; Peranginangin, 2018). Maceration has drawbacks regarding yield and efficiency, so it takes at least three days to macerate, so it will be challenging to achieve large-scale production quickly, especially within an industrial context.

Hot kinetic maceration alleviates the hindrance associated with traditional maceration techniques by utilizing kinetic energy to enhance mass transfer. This results in more frequent interaction between the material and the solvent.

Additionally, heat helps soften and break down the material's cell walls, allowing plant compounds to diffuse more effectively into the solvent. This extraction method can be performed using a hotplate magnetic stirrer, which is more productive than a water bath shaker (Sarkar & Ghosh, 2017). Improving the extraction process efficiency is necessary to optimize temperature and kinetics.

However, temperature optimization is more prone to reduce effectiveness, especially antioxidant power, because of the thermolabile nature of the numeric pigment. Therefore, kinetic optimization is carried out (Putra *et al.*, 2021). The effect of kinetic variation on IC_{50} and yield has been carried out on several types of plants where the optimal stirring speed was 400 rpm for orange peel samples, 600 rpm for *Copaifera langsdorffii* samples, and 800 rpm for seaweed samples (Costa-Machado, Bastos and de Freitas, 2013; González *et al.*, 2016; Haya, Bentahar and Trari, 2019).

In addition to kinetics, IC_{50} is also influenced by the variety and condition of the materials used. Red Ginger is proven to have more substantial antioxidant power than white ginger, and dried ginger has better antioxidant power than wet ginger, which still contains plenteous water (Ghasemzadeh *et al.*, 2010; Munadi, 2018; Mao *et al.*, 2019).

Based on this explanation, a study was conducted to determine how increasing kinetic rate affects IC_{50} and yield on the combination extract of angkak and red ginger using the kinetic hot maceration extraction method with variations in rotational speed, specifically 400, 600, and 800 rpm. This study also proved the benefits of ethnomedicine of Angkak and red ginger through antioxidant value; the IC_{50} testing was carried out using the DPPH method, which operates based on the principle of complexing of DPPH radical solutions with hydrogen atom donors or other radicals. The interaction results in the formation of non-radical DPPH, leading to a subsequent reduction or loss of absorbance. The reduction in the number of DPPH radicals provides an approximate index of the ability of the test compound to trap radicals (Francenia Santos-Sánchez *et al.*, 2019). It is hoped that the results of this research will be helpful in drug processing in society by producing extracts with large yields and maximum antioxidant value with minimal time and also reduce production costs because the equipment is simple and does not require many containers to extract large quantities so that several extractions can be carried out in one day. It is also hoped that this research will help develop the ethnomedicine of red ginger and angkak so that people will not be confused about using them.

METHOD

Research Tools and Materials

The red ginger is a dry powder obtained from CV Agradaya Indonesia, Yogyakarta, Indonesia, with HACCP, Halal, and BPOM (Indonesian Food and Drugs Administration) certification. The angkak is red rice obtained from the BPOM-certified SU Brand imported by PT Global Buana Mandiri, Jakarta, Indonesia. Other ingredients include 96% ethanol (working standard) as the extracting solvent, DPPH powder for the antioxidant test (TCI Lot no WZ4DO-LQ), silica 60 F254 as the stationary phase of TLC (Supelco serial number 1.05554.0001), toluene (pro analysis), acetone (pro analysis), ethyl acetate (pro analysis), acetic acid (pro analysis), and distilled water (working standard) as the mobile phase of TLC. Vanillin sulfate 3% was used as an antidote for red ginger TLC spots. Details regarding the research flow are shown in Figure 1.

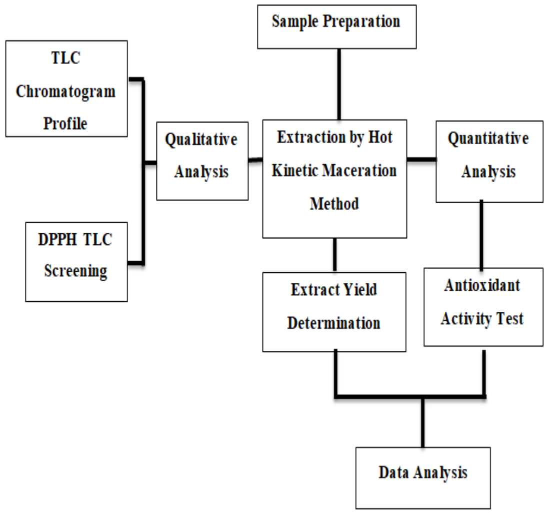

Figure 1.
Research Flowchart
Source: Research documentation

The tools used are type 2450 UV-Vis spectrophotometry, hotplate magnetic stirrer, analytical balance, rotary evaporator, sonicator, micropipette (dlab), oven, and 366 nm UV sight.

Extraction by Hot Kinetic Maceration Method

Angkak was first prepared by mashing it with a blender and sifting it with sieve no. 40. The extraction step modifies the research by Gutiérrez *et al.* (2014), namely red ginger powder (35 g) and angkak (15 g) combined and extracted in ethanol with a ratio of 1:20 using a hotplate magnetic stirrer at 60°C for 2 hours. The macerate is then allowed to settle and filtered with a Büchner funnel (Gutiérrez *et al.*, 2014).

The Büchner filtrate was thickened using a rotary evaporator, which was dried in an oven at 50°C for three days. Each variable, namely the stirring speed of 400, 600, and 800 rpm, was carried out in the same steps three times following the procedure used by Yulia Senja *et al*. until each variable resulted in three viscous extracts, which means that there were three extracts for the 400 rpm variable, there were three extracts for variable 600 rpm, and there are three more extracts for the variable 800 rpm (Yulia Senja *et al.*, 2014).

Extract Yield Determination

The determination of extract yield follows the procedure stated in the Indonesian Herbal Pharmacopoeia by dividing the final extract's weight in g with the total dry sample powder's weighting (Ministry of Health Services Indonesian Republic, 2017).

Chromatogram Profile Determination with TLC

The chromatogram profile test was divided into two parts: one analyzing the red ginger profile within the extract and the other explicitly targeting the extract's red ginger profile. The TLC procedure follows the guidelines outlined in the Indonesian Herbal Pharmacopoeia, with some modifications from the Thin Layer Chromatography book. It involves using ethanol as the sample solvent and a mobile phase consisting of toluene and acetone in a 9:1 ratio for analyzing the specific chromatogram profile of red ginger material.

Additionally, for the specific chromatogram profile of angkak, the mobile phase consists of ethyl acetate, distilled water, and methanol in a 7:1:1 ratio, along with adding one drop of acetic acid. (Wall, 2005; Ministry of Health Services Indonesian Republic, 2017). The extract chromatogram profiles were compared with the literature. Separation spots were observed at UV 254 nm and 366 nm, visible wavelengths, and specifically for the red ginger ingredient-specific TLC, a vanillin sulfate spot enhancer was added.

Antioxidant Activity Test

Preliminary screening of antioxidant compounds using TLC adheres to the procedures stated in the Indonesian Herbal Pharmacopoeia, and for the mobile phase, the TLC profile chromatogram is adhered to (Ministry of Health Services Indonesian Republic, 2017). Antioxidant compounds were identified with DPPH sprayers.

The antioxidant activity of extracts is determined through the DPPH method, involving a comparison with vitamin C. Vitamin C and extracts were dissolved in ethanol and diluted in 5 concentrations, namely 3, 5, 7, 9, and 11 ppm for vitamin C, and 30, 60, 90, 120, 150 ppm for extract. Each series was added with 45 ppm DPPH with a ratio of 1:2, followed by incubation for 30 minutes in a tightly closed container and protected from light. Blanks were made from a 2:1 ratio of 45 ppm DPPH and ethanol. The absorbance of the blank and concentration series was measured at a wavelength of 517 nm with triple measurements for each concentration series.

Calculation of percent inhibition using the formula (Pandiangan *et al.*, 2020):

$$\frac{(Abs\ \underline{x}\ DPPH - Abs\ \underline{x}\ sampel)}{Abs\ \underline{x}\ DPPH} x100\% \qquad (1)$$

Then, plotted the concentration against % inhibition and continued with the calculation of IC_{50} using the formula (Pandiangan *et al.*, 2020):

$$x = \frac{(y - a)}{b} \qquad (2)$$

where y is 50 and the values b, x, a, are obtained from the equation y=bx+a

Data analysis

Data were analyzed for homogeneity using the Levene Test ($p > 0.05$) and normality using Shapiro-Wilk ($p > 0.05$), which was then followed by an ANOVA test to see if the data were parametric. At the same time, if the data were nonparametric, it was continued with the Kruskal Wallis test to see whether there was significance ($p < 0.05$). A posthoc test on parametric data followed data showing significance. Overall, data analysis was performed using SPSS.

Comparison Step

For comparison, separate extraction was carried out at the optimal rotation speed from the results of the above research on single red ginger using 35 g of red ginger powder and single angkak using 15 g of angkak powder in 1000 mL of ethanol at the same time and temperature as the combined extract. After that, the yield, klt, and IC_{50} values were also carried out, which were the same step as for the combined extract.

RESULTS AND DISCUSSION

Extraction

Both single angkak and combination extract obtained are viscous because the starch in brown rice gives a mucilage-like structure. Both single red ginger and combination extracts are also slightly oily due to the essential oil in red ginger (Cicero *et al.*, 2019; Tritanti & Pranita, 2019). The dark red color of the combination extract is due to a mixture of red pigment in angkak and the brownish-yellow color of single ginger (Hasim *et al.*, 2018; Tritanti & Pranita, 2019). Ginger essential oil has a characteristic spicy odor (Tritanti & Pranita, 2019).

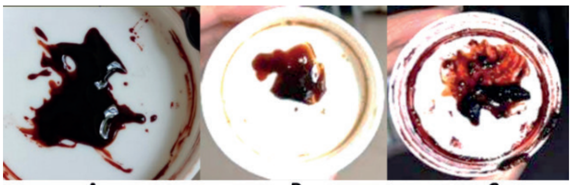

Figure 2.
Appearance of Extracts: (A) Combination extract; (B) Single red ginger extract; (C) Single angkak extract
Source: Research documentation

Extract Yield

Properly selecting fixed variables determines the optimal yield and antioxidant activity achievement through kinetic heat extraction. Choosing 120 minutes led to an extended sample exposure to the solvent, providing sufficient time for the compounds to migrate into the solvent. The 60°C is selected because, at that temperature, gingerol degrades into *shogaol*, which has more substantial antioxidant power, and also because the components of the yellow, red, and orange pigments in angkak, which contribute antioxidants, are more soluble at high temperatures (Rahmadani *et al.*, 2008; Che Sulaiman *et al.*, 2017; Putra *et al.*, 2021).

The use of ethanol is supported by a 1:20 material and solvent ratio, which increases the concentration gradient, thereby increasing the diffusion rate of the extracted substance into the solvent (Do *et al.*, 2014; Predescu *et al.*, 2016). Ethanol also has semipolar properties, making it suitable for *gingerols* and *shogaols*, which have good solubility in organic solvents (non-polar) (Abu *et al.*, 2017; Hasim *et al.*, 2018).

The highest yield percentage was obtained with a stirring speed of 400 rpm, averaging 15.933 ± 3.4771% (Table 1). The analysis results showed a significant difference in % yield between 400 rpm to 600 and 800 rpm. That is, there will be a decrease in yield gain after 400 rpm.

The decrease in yield percentage with increasing stirring speed is different from the results of research conducted on samples of orange peel, *Copaifera langsdorffii*, and seaweed, where in these studies, it was observed that as the stirring speed increased, the percentage yield also increased. This increase in yield percentage is correlated to the diffusion mechanism in the dissolved material affected by the relative velocity of the particles in the fluid. In this study, the velocity is caused by stirring the stirrer bar. Diffusion occurs in the area surrounding the solid with a slow fluid velocity. When the relative velocity of the particles in a fluid increases due to agitation, it can carry the solid surface further away from the liquid surface (Susanti *et al.*, 2020; Andersson *et al.*, 2022).

The speed of solid movement depends on the particle size because it will affect the ability of fluidization when a solid is in contact with a fluid and will have fluid-like properties. The smaller the particle size, the smaller the minimum fluidization velocity, which is the minimum speed required for solids to behave like fluids, and this means that small particle sizes will be easy to suspend in fluids. Previous research on the samples did not mention the fineness of the extracted particle size, so the difference in results for this study could also be caused by the particle size, which contributed to the decrease in diffusion due to particles' easy movement with the fluid (Timsina *et al.*, 2019).

One of the components that can be present in the extract due to diffusion is the outcome of cell wall breakdown. These cell wall fragments are one of the ballast substances, namely impurity compounds commonly found in unpurified extracts (crude extracts) (Amaliah *et al.*, 2019).

The cell wall can undergo lysis due to ethanol solvent diffusing into the cell, where this diffusion will cause the cell to expand because it contains solvent and then rupture. Thus, it can be explained that the use of 400 rpm in this study provides good diffusion power because it increases the contact between the cell and the solvent so that with the support of temperature at 60^{o}C, it can increase the ability to lyse the cell wall, and causes an increase in yield compared to 600 and 800 rpm (Nemazifard *et al.*, 2017; Chairunnisa *et al.*, 2019).

The results of the comparison between the yields of the combined extract and the single extract of each ingredient showed a significant difference at 400 rpm with the single extract of angkak and red ginger. In contrast, at 600 and 800 rpm, there is only a significant difference for the single extract of angkak.

TLC profile

The chromatogram test was carried out separately based on the component elements of the extract, namely red ginger, and angkak, where the mixed extract was eluted in 2 separate chambers. The eluent used in each chamber is specific for each material, with a special eluent for red ginger and a separate eluent for angkak because the compounds in each material have different polarities. In contrast, the compounds in angkak tend to be polar (Basuny and Abdel-Raheam, 2020; Amalia and Sabila, 2021). To effectively separate the compounds in red ginger and angkak extracts, a specific eluent combination tailored to their respective polarities was used. For the red ginger extract, which contains non-polar compounds, the eluent is chosen accordingly.

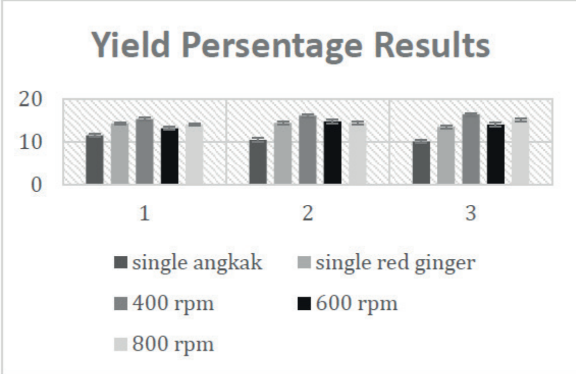

Figure 3.
Yield Percentage Result
Source: Research documentation

The angkak extract consists of a mixture of ethyl acetate, water, and methanol in a 7:1:1 ratio. Acetic acid is added to this mixture to enhance separation. The acid prevents tailing and ensures sharper, more distinct bands by ionizing polar compounds, particularly the pigment compounds in angkak, which are highly sensitive to acidic pH levels. (Wall, 2005; Ministry of Health Services Indonesian Republic, 2017).

Up to five distinct stains are visible on the specific chromatogram profile of the red ginger material within the extract. The number depends on where it grows, so red ginger obtained from various places can produce various stains on the chromatogram (Marwati *et al.*, 2021). Red ginger is known to contain the main phenolic compounds, namely gingerol, shogaol, and paradol, as well as steroids and essential oils, which can react with the vanillin sulfate agent to give a specific blue color, which in this study, blue to purplish color was the result (Merck, 1980; The United States Pharmacopeial Convention, 2010; Mao *et al.*, 2019; Amalia & Sabila, 2021; Thomas *et al.*, 2021). Referring to studies using HPTLC with the same fluent and mobile phase as this study, it can be identified that it is 6-gingerol at rf 0.26 for both single red ginger extract and combination extract (Kumar *et al.*, 2022).

The 6-gingerol compound is also more polar because it is not carried away by a non-polar element and is retained on a polar plate, so it has a low value. Two stains have rf similarities with ginger TLC in the Indonesian Herbal Pharmacopoeia (FHI) literature, specifically a stain with rf 0.42 in single red ginger extract and a stain with rf 0.4 in combination extract, which is similar to rf 0.37 in FHI, and a stain with rf 0.48 which is similar to rf 0.45 in FHI (Ministry of Health Services Indonesian Republic, 2017). It also can be seen that the stains in single red ginger extract are like those in combination extract overall. However, these compounds are more accessible to separate in a single extract. It can be seen from a stain with rf 0.5 in the single extract that cannot be found in combination extract, and this can be caused by another compound in angkak that have similar polarity (a stain with rf 0.54 in Figures 5 and 6) with that compound in red ginger (Matysik *et al.*, 2016). However, the specific compound in the stain cannot be ascertained because it requires a comparison.

The specific TLC chromatogram profile of the angkak material in the extract shows that the angkak contains two pigments, as indicated by the color of the separated stain: one red pigment and one yellow pigment. The results are analogous to the literature that angkak contains red and yellow pigments, although, in this study, no orange pigment was found (Basuny & Abdel-Raheam, 2020). Based on the TLC results in this study, it is understood that yellow pigment is more non-polar than red pigment because a more non-polar element easily carries it away and is retained less on a polar plate, so it has a higher rf value.

The stain from both single angkak and combination TLC also have a similar problem with previous red ginger TLC, which can be seen from a stain with rf 0.06 in single angkak extract that cannot be found in combination extract, and this can be caused by another compound in red ginger that have similar polarity (a stain with rf 0.1 in figure 3 and 4) with that compound in angkak (Matysik *et al.*, 2016).

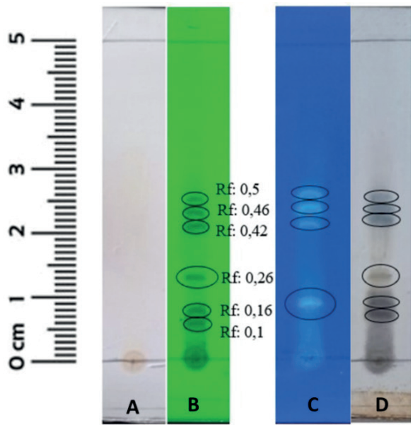

Figure 4.
Chromatogram Profile of Red Ginger (Ingredients in the Single Red Ginger Extract, where Stationary phase = Silica Gel 60 F254; Mobile phase = Toluene: acetone (9:1): (A) Plate at a visible wavelength (before observing with the spot sight); (B) The plate, subsequently being observed under a 254 nm UV spotting viewer; (C) The plate subsequently being observed under the 366 nm UV spotting viewer; (D) The plate subsequently being sprayed with vanillin sulfate reagent)
Source: Research documentation

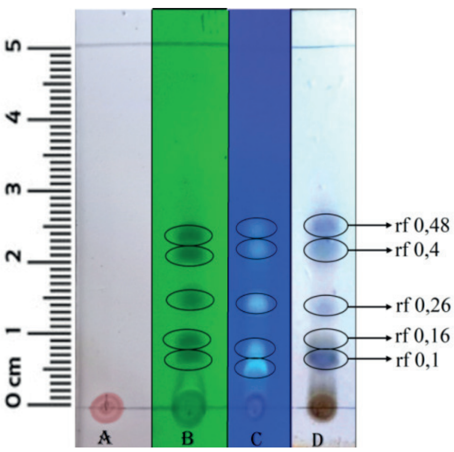

Figure 5.
Chromatogram Profile of Red Ginger (Ingredients in the Combination Extract of Red Ginger and Angkak, where Stationary phase = Silica Gel 60 F254; Mobile phase = Toluene: acetone (9:1): (A) Plate at a visible wavelength (before observing with the spot sight); (B) The plate, subsequently being observed under a 254 nm UV spotting viewer; (C) The plate subsequently being observed under the 366 nm UV spotting viewer; (D) The plate subsequently being sprayed with vanillin sulfate reagent)
Source: Research documentation

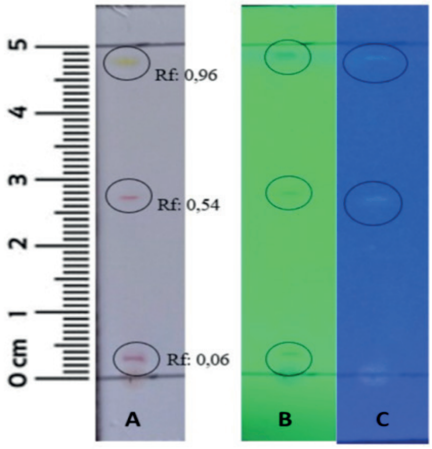

Figure 6.
Chromatogram Profile of Angkak (Ingredients in Single Angkak Extract, where Stationary phase = Silica Gel 60 F254; Mobile phase = Ethyl acetate: methanol: distilled water (7:1:1) plus 1 drop of acetic acid: (A) Plate at a visible wavelength (before observing with the spot sight); (B) The plate, after being observed under a 254 nm UV spotting viewer; (C) The plate, after being observed under the 366 nm UV spotting viewer)
Source: Research documentation

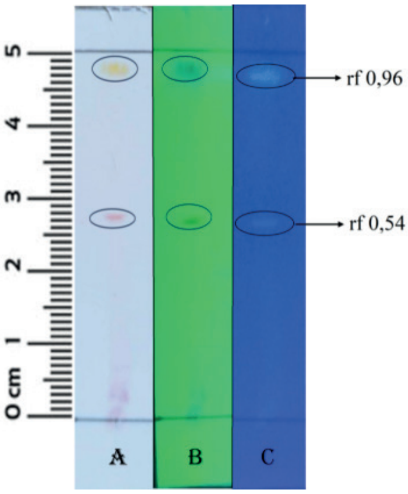

Figure 7.
Chromatogram Profile of Angkak (Ingredients in Red Ginger-Angkak Extract Combination, where Stationary phase = Silica Gel 60 F254; Mobile phase = Ethyl acetate: methanol: distilled water (7:1:1) plus 1 drop of acetic acid: (A) Plate at a visible wavelength (before observing with the spot sight); (B) The plate, after being observed under a 254 nm UV spotting viewer; (C) The plate, after being observed under the 366 nm UV spotting viewer)
Source: Research documentation

Extract Antioxidant Activity

Preliminary DPPH screening, as seen in Figure 3, shows that there are antioxidant compounds in the angkak and red ginger, which can be seen from the change in the stain to yellowish, and the compounds contributing to these antioxidants are the same compounds identified on the chromatogram profile.

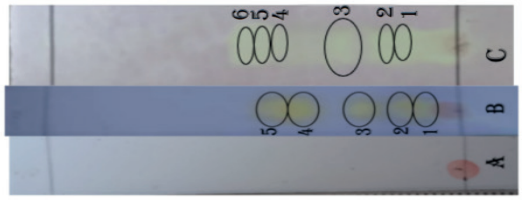

Figure 8.
Screening of Antioxidant Activity of Red Ginger Materials
(where Stationary phase= Silica Gel 60 F254; Mobile phase = Toluene: acetone (9:1): (A) Plate at a visible wavelength (before observing with the spot sight); (B) Plate after being sprayed with DPPH 45 ppm (combination extract); (C) Plate after being sprayed with DPPH 45 ppm (single red ginger extract))
Source: Research documentation

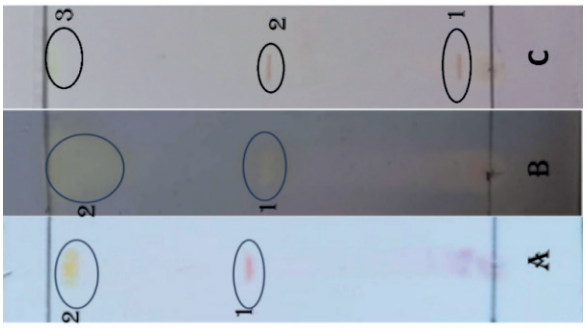

Figure 9.
Screening of Antioxidant Activity of Angkak Ingredients
(where Stationary phase= Silica Gel 60 F254, Mobile phase= Ethyl acetate: methanol: distilled water (7:1:1) plus 1 drop of acetic acid: (A) Plate at a visible wavelength (before observing with the spot sight); (B) Plate after being sprayed with DPPH 45 ppm (combination extract); (C) Plate after being sprayed with DPPH 45 ppm (single red ginger extract))
Source: Research documentation

The highest IC_{50} value is at 400 rpm with an average of 103.76 ± 10.032 ppm. There was no significant difference between the variations in rpm and IC_{50}, which means there was no correlation between the two, and this is different from studies conducted on samples of orange peel, *Copaifera langsdorffii*, and seaweed, where in this study, there was an increase in antioxidant activity when the spin speed was increased. This increase in antioxidant activity might be attributed to the maximum extraction of antioxidant compounds at 400 rpm, so IC_{50} will also be at its paramount condition, and an increase in stirring speed will not impact it. This condition is also experienced by studies using coffee skin samples and *Withania somnifera*, where the IC_{50} will increase by adding the variable degree (Sushma *et al.*, 2014; Dhanani *et al.*, 2017; Kusumocahyo *et al.*, 2020).

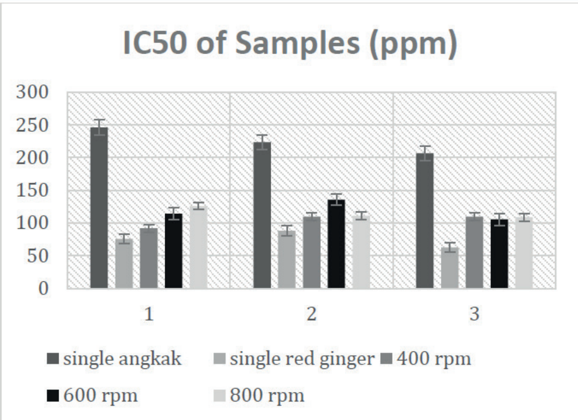

Figure 9.
Results of Antioxidant Activity Assessment
Source: Research documentation

The IC_{50} value, which does not correlate with rotational speed, means that IC_{50} also does not correlate with the yield percentage, which is different from research on samples of orange peel, *Copaifera langsdorffii*, and seaweed, a correlation between IC_{50} and percent yield were identified. The rotational speed because the results of cell wall breakdown carried in the extract due to cell lysis during diffusion do not have antioxidant activity, a cellulose compound that has antioxidant activity (AG *et al.*, 2020). This rotational speed causes the increase in yield obtained at 400 rpm, which does not correspond to a significantly higher IC_{50} value. It has the same value as the IC_{50} extraction at 600 and 800 rpm, with a lower yield percentage (Manuel *et al.*, 2013; Dhanani *et al.*, 2017; Zaidiyah *et al.*, 2021).

The ic50 value of the whole combination extract is higher than the single angkak extract but lower than the single red ginger extract. This result could be caused by the quality of the angkak needing to be more optimal, where from the measurement results, it is known that the angkak has a medium antioxidant value. The presence of orange pigment and the levels of other pigments are influenced by the type of *Monascus purpureus* strain, inoculum content, temperature, fermentation time, and type of rice (Hamdiyati *et al.*, 2016; Marič *et al.*, 2019; Saithong *et al.*, 2019).

In addition, how the product is stored before extraction also affects the compounds that contribute to antioxidants in angkak, where red pigment is degraded at high temperatures. Angkak pigment has a strong tendency to absorb visible light and radiant energy from lamps, which causes a reduction in stability when exposed to more light. After 24 hours, storage errors damage antioxidant-contributing compounds (Putra *et al.*, 2021). Meanwhile, red ginger has a substantial antioxidant value and can act synergistically with angkak. (Skroza *et al.*, 2022).

CONCLUSION

Kinetic, the stirring speed affects the percent yield but not the antioxidant activity. Stirring speed that is too high causes a decrease in yield percent. The best % yield and antioxidant activity were obtained from a stirring speed of 400 rpm with an average value of 15.933 ± 3.4771% and 103.76 ±10.032 ppm. The combination of angkak and red ginger can work synergistically, although a very strong IC_{50} was not obtained in this study.

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