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Antioxidant Activity Comparison of *Jamu Cekok* and Its Individual Herbal Components Using the DPPH Method

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Abstract

Jamu cekok is a traditional Indonesian herbal drink commonly consumed by children to increase appetite. It is made from a combination of several plants, including *Curcuma zanthorrhiza (C. zanthorrhiza), Curcuma longa (C. longa), Zingiber officinale (Z. officinalle), and Kaemferia galanga (K. galanga).* The combination of these plants potentially enhanced the antioxidant effects due to the synergism or antagonism interaction among the constituent herbs. This research aimed to determine the antioxidant activity of jamu cekok and compare it with the antioxidant activity of its individual herbal components. Methods were started with the extraction of jamu cekok using ethanol as the solvent, prepared from a 1:1:1:1 ratio of *C. zanthorrhiza, C. longa, Z. officinale, and K. galanga extract.* The resulting extracts were tested using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay and phytochemical screening. Analysis revealed that *jamu cekok* extract contains alkaloids, flavonoids, and saponins. Turmeric, ginger, and galangal exhibited strong antioxidant activity with IC_{50} values of 96.158 ppm, 87.040 ppm, and 83.855 ppm, respectively. Curcuma showed moderate antioxidant activity with an IC_{50} value of 112.227 ppm. *Jamu cekok* exhibits very strong antioxidant activity (IC_{50} = 46.904 ppm), surpassing the activity of its individual herbal components. The synergistic interaction among bioactive compounds, particularly alkaloids, flavonoids, and saponins, is presumed to contribute to the enhanced antioxidant potential of jamu cekok. These results support its role as a natural antioxidant source and provide a scientific basis for its traditional use in health promotion.

Keywords: Antioxidant; DPPH; Extract; Free Radical Scavengers.

INTRODUCTION

Traditional herbal medicine has long played a significant role in the healthcare systems of many cultures, particularly in Indonesia, where remedies such as jamu cekok are widely used to manage various pediatric health issues (Adiyasa et al., 2021). Jamu cekok, a traditional herbal concoction, is primarily administered to children to address conditions such as loss of appetite, helminthiasis, cough, common cold, and abdominal bloating. The term “cekok” originates from the Javanese language, referring to the method of forcibly administering the herbal mixture into a child’s mouth, a practice that has been passed down through generations. Feeding difficulties in young children represent a prevalent parental concern, particularly during the critical developmental period between 1 and 3 years of age, commonly referred to as the preschool years. This phase is frequently associated with a notable deceleration in physical growth relative to infancy, which may contribute to reduced appetite and feeding challenges (Sunarmi & Suhendriyo, 2023). Persistent feeding difficulties are significant concern, as they can compromise adequate nutritional intake and subsequently impact both physical growth and cognitive development in children (Nurfieni et al., 2017). The main ingredients of *jamu cekok* typically include a blend of medicinal plants such as papaya leaves (*Carica papaya L.),* Javanese ginger (*Curcuma xanthorrhiza Roxb*.), black turmeric (*Curcuma aeruginosa Roxb*.), turmeric (*Curcuma longa L*.), fennel (*Foeniculum vulgareMill*.), aromatic ginger (*Kaempferia galanga L*.), and green chiretta (*Andrographis paniculataNees*.)(Zulaikha et al., 2021).

Empirical evidence suggests that jamu cekok may contribute to improved appetite and weight gain in children, which is commonly attributed to the phytochemical constituents and antioxidant properties of its herbal components (Bagus Wicaksono et al., 2017; Marni & Retno, 2015). Phytochemicals such as flavonoids, curcuminoids, and other bioactive compounds are known to exhibit antioxidant activities that may help mitigate oxidative stress and support overall health (Bagus Wicaksono et al., 2017). The interaction of multiple herbal ingredients can result in

synergistic or antagonistic effects on antioxidant activity, depending on the specific plant combinations and their ratios (Marianne et al., 2018; Pusmarani et al., 2024). However, scientific data comparing the antioxidant activities of the complete jamu cekok mixture with those of its individual herbal constituents remain limited.

Despite its widespread use, the pharmacological basis of jamu cekok's efficacy, particularly its antioxidant activity, has not been thoroughly investigated. The DPPH (1,2-diphenyl-2-picrylhydrazyl) assay is a well-established method for measuring free radical scavenging capacity and is widely used to evaluate the antioxidant potential of herbal extracts (Amin et al., 2016; Wulan et al., 2019). In light of these considerations, this study aims to analyze and compare the antioxidant activity of jamu cekok and its individual herbal components using the DPPH method. The findings will provide valuable insights into the potential mechanisms underlying the traditional use of jamu cekok and contribute to a more evidence-based understanding of its health benefits.

MATERIALS AND METHODS

Sample Preparation

C. zanthorrhiza, C. longa, Z. officinale, and K. galanga were obtained from the Flamboyan traditional market in South Pontianak, West Kalimantan, Indonesia. The collected rhizomes were thoroughly cleaned and rinsed with clean water, then drained. Each sample was sliced to a thickness of 3–5 mm and dried in an oven at 50°C. After drying, the simplicia were ground using a blender and sieved to obtain a fine powder. The resulting powder was weighed and stored in tightly sealed containers.

Procedures

Maceration

A multi-stage maceration process was conducted using 96% ethanol as the solvent. In separate containers, 100 g each of temulawak, turmeric, kencur, and ginger simplicia were submerged in 1 L of ethanol. Maceration proceeded for 3 days, with the ethanol replaced every 24 hours and the mixtures stirred occasionally. After each solvent change, the filtrate was collected by filtration through filter paper, and all filtrates. All filtrates was concentrated using a rotary evaporator at 50°C to obtain a thick extract, which was stored in a sealed container (Abubakar & Haque, 2020). The extract yield was calculated using the following equation:

$$Yield\ (\%) = \frac{Weight\ of\ the\ obtained\ extract\ (gram)}{Weight\ of\ the\ simplicia\ before\ ectraction\ (gram)} x100\%$$

Phytochemical Analysis

Extracts of *C. zanthorrhiza, C. longa, Z. officinale, and K. galanga* were subjected to phytochemical screening to identify secondary metabolites, including alkaloids, saponins, tannins, and flavonoids, according to standard procedures (Hanani MS, 2015; Harbone, 1998).

Alkaloids determination: Several milligrams of extract were dissolved in 9 mL of distilled water and 1 mL of 2N HCl, then heated in a water bath for 2 minutes and allowed to cool. The solution was filtered. Two milliliters of the filtrate were treated with 2 drops each of Wagner's, Dragendorff's, and Mayer's reagents. A positive result was indicated by the formation of a brown precipitate with Wagner's reagent, an orange-brown precipitate with Dragendorff's reagent, and a white or yellow precipitate with Mayer's reagent.

Flavonoids determination: Flavonoid content was assessed using a qualitative color test. The extract was dissolved in 5 mL of 96% ethanol. To 2 mL of this solution, 0.1 g of magnesium powder and 10 drops of concentrated hydrochloric acid were added, and the mixture was gently shaken. Formation of a red to reddish-purple color indicated a positive result for flavonoids.

Saponins determination: Several milligrams of extract were placed in a test tube with 10 mL of hot distilled water. After cooling, the mixture was vigorously shaken for 10 seconds. The formation of stable foam with a height of 1–10 cm that persisted for at least 10 minutes was considered a positive result for saponins. To confirm the presence of saponins, one drop of 2N HCl was added; the foam remained stable and did not disappear.

Tannins determination: Several milligrams of extract were dissolved in 5 mL of hot water, stirred, and cooled. The mixture was centrifuged, and the supernatant was decanted and filtered. Tannins were detected by adding 2 drops of 3% $FeCl_3$ to 1 mL of filtrate; a positive result was indicated by the appearance of a greenish-violet color.

Antioxidant Assay

Antioxidant activity was assessed at five different concentrations for each sample extract, selected based on inhibition percentages (ranging from below 50% to above 50%). The antioxidant activity test of ethanol extracts of *C. zanthorrhiza, C. longa, Z. officinale, K. galanga,* and jamu cekok was conducted at five different concentrations. Quercetin was tested at five concentrations as the positive control, and ethanol served

as the negative control. For the assay, 3 mL of sample was mixed with 2 mL of DPPH solution. The mixture was incubated in the dark for 30 minutes. Absorbance was measured at a wavelength of 512.6 nm using a UV-Vis spectrophotometer (Shimadzu, Model UV-2450) (Gulcin et al., 2023; Suhartati, 2017). All measurements were performed in triplicate. The percentage of DPPH free radical inhibition was calculated using the following equation:

$$\%inhibisi = \frac{Sampel\ Absorbance - Control\ Absorbance}{Control\ Absorbance}\ x\ 100\%$$

The antioxidant activity of a compound is commonly evaluated based on its IC_{50} value. The IC_{50}, or inhibition concentration, represents the concentration required to inhibit 50% of DPPH free radicals. The IC_{50} value was determined using a linear regression equation correlating test solution concentration with the percentage of DPPH inhibition (Gulcin et al., 2023). An IC_{50} value less than 50 ppm indicates very strong activity, 50-100 ppm is considering strong, 101-150 ppm is moderate, 151-200 ppm is weak and a value above 200 ppm is considered very weak. (Marianne et al., 2018)

Data analysis

All data obtained were processed and analyzed descriptively using Microsoft Excel.

RESULTS AND DISCUSSION

Plant extract

All dried sample were analyzed for moisture content (Table 1).

Table 1. Moisture content of temulawak, turmeric, ginger and kencur.

Plants	Wet weight (g)	Dry weight (g)	Moisture content (%)
C. zanthorrhiza	255,21	133,16	49,25
C. longa	290,31	126,89	57,75
Z. officinale	696,01	246,38	65,29
K. galanga	613,92	228,50	63,54

The moisture content of the powdered materials from *C. zanthorrhiza*, *C. longa*, *Z. officinale*, and *K. galanga* was found to be relatively high, measured at 49.25%, 57.75%, 65.29%, and 63.55%, respectively. The extraction method used in this study was maceration with 96% ethanol as the solvent. The maceration results were then evaporated to yield a thick extract, with the percentage yield (% yield) (Table 2).

Table 2. Yield persentage of temulawak, turmeric, ginger and kencur.

Plants	Simplicia (g)	Extract (g)	Yield (%)
C. zanthorrhiza	100	27,4	27,4
C. longa	100	18,2	18,2
Z. officinale	100	23,6	23,6
K. galanga	100	36,7	36,7

K. galanga exhibited the highest yield at 36,7%, while *C. longa* produced the lowest yield at 18,2%.

Phytochemical screening

Phytochemical screening identifies active compounds that may have therapeutic effects in plant-based drug development and helps to understand the mechanisms of action as well as the biological interactions of the identified compounds. In this study, the secondary metabolites examined in the ethanol extracts of *C. zanthorrhiza*, *C. longa*, *Z. officinale*, *K. galanga*, and the herbal mixture "jamu cekok" included alkaloids, saponins, flavonoids, and tannins. (Table 3).

Table 3. Phytochemical screening of temulawak, turmeric, ginger and kencur.

Assay	Reagent	Ethanol extracts				
		C. zanthorrhiza	*C. longa*	*Z. officinale*	*K. galanga*	Jamu cekok
Alkaloid	Wagner	+	+	+	+	+
	Mayer	+	+	+	+	+
	Dragendorff	+	+	+	+	+
Saponin	Aquadest	-	-	+	-	+
Flavonoid	Mg + Concentrated HCl	-	+	-	-	+
Tannin	$FeCl_3$ 3%	-	-	-	-	-

Description:
+ : Detected, - : Undetected

C. zanthorrhiza, *C. longa*, *Z. Officinale*, *K. galanga*, and the herbal mixture "jamu cekok" tested positive for alkaloids (using Wagner, Mayer, and Dragendorff reagents) and negative for tannins. For saponins, only the

ginger extract and the "jamu cekok" extract tested positive, while flavonoids were detected only in the *C. longa* extract and the "jamu cekok" extract.

DPPH Scavenging Activity

A lower IC_{50} value indicates a stronger ability of the compound to inhibit free radicals. Conversely, a higher IC_{50} value suggests lower antioxidant activity. The IC_{50} value is obtained from the linear regression equation between the concentration of the test solution and the percentage inhibition of DPPH.

Table 4. Inhibitory Concentration (IC_{50}) Value of Antioxidant DPPH Scavenging Activities of Temulawak, Ginger, Curcuma, Kencur, Jamu Cekok Extract, Quercetin and Negative Control.

Samples	Concentration	Absorbance	Inhibiton (%)	Linear equation	IC_{50} (ppm)	Category
C. zanthorrhiza	50 ppm	0,606	20,59	y = 0,6575x – 13,224	96,158	Strong
	70 ppm	0,524	31,34			
	80 ppm	0,480	37,02			
	90 ppm	0,433	43,18			
	100 ppm	0,356	53,32			
Z. Officinale	50 ppm	0,475	37,67	y = 0,3775x + 20,621	87,040	Strong
	60 ppm	0,453	40,60			
	70 ppm	0,422	44,71			
	80 ppm	0,406	46,81			
	90 ppm	0,370	51,44			
C. longa	50 ppm	0,472	38,07	y = 0,4272x + 16,177	83,855	Strong
	60 ppm	0,447	41,43			
	80 ppm	0,380	50,22			
	90 ppm	0,353	53,76			
	100 ppm	0,307	59,75			
K. galanga	50 ppm	0,614	19,49	y = 0,483x – 4,23	112,277	Moderate
	60 ppm	0,578	24,21			
	80 ppm	0,503	34,05			
	90 ppm	0,397	49,90			
	100 ppm	0,372	51,22			
Jamu cekok	50 ppm	0,383	49,83	y = 0,6228x + 20,788	46,904	Very strong
	60 ppm	0,310	59,31			
	70 ppm	0,253	66,83			
	80 ppm	0,218	71,46			
	100 ppm	0,139	81,77			
Quercetin	2 ppm	0,754	1,09	y = 7,004x – 13,038	9,000	Very strong
	4 ppm	0,652	14,47			
	6 ppm	0,531	30,33			
	8 ppm	0,447	41,35			
	10 ppm	0,323	57,69			
Negative Control		0,763				

The ethanol extracts of temulawak, ginger, and turmeric exhibited strong antioxidant activity with IC_{50} values of 96.158 ppm, 87.040 ppm, and 83.855 ppm, respectively. In comparison, kencur extract showed moderate activity with an IC_{50} of 112.277 ppm. The ethanol extract of jamu cekok, which is a combination of the four rhizomes mentioned above, demonstrated the strongest antioxidant activity with an IC_{50} value of 46.904 ppm. As a reference standard, quercetin exhibited a much stronger antioxidant activity with an IC_{50} value of 9 ppm.

Discussion

Maceration and phytochemical screening

Moisture content determination measures the water present in a sample and is crucial because it affects the solvent concentration during maceration. High moisture lowers the effective solvent concentration due to dilution, while low moisture facilitates extraction by allowing the solvent to penetrate cell walls without water interference (Wijaya et al., 2022). Moisture also impacts sample shelf life by influencing microbial activity. Low moisture ensures optimal stability and inhibits microbial growth, whereas high moisture increases susceptibility to microbial degradation and fungal growth (Korua, 2020). According to the Indonesian Herbal Pharmacopoeia (2008) and Minister of Health Decree No. 661/Menkes/SK/VII/1994, the maximum moisture content for simplicia is 10% (Manalu et al., 2012). However, Table 1. shows that *C. zanthorrhiza*, *Z. officinale*, *C. longa*, and *K. galanga* simplicia have high moisture levels of 49.25%, 57.75%, 65.29%, and

63.55%, respectively, indicating inadequate drying. Inadequate drying conditions, such as improper temperature and humidity, likely cause this high moisture. During drying, rhizomes shrink due to water evaporation and heat-induced cell stress, altering their shape and size. Excessive shrinkage can degrade product quality by damaging active compounds (Manalu et al., 2012).

Rhizomes of *C. zanthorrhiza*, *Z. officinale*, *C. longa*, and *K. galanga* were extracted using maceration with 96% ethanol at room temperature to isolate secondary metabolites. Maceration, a cold extraction method, was chosen to preserve antioxidant compounds, while 96% ethanol, a polar solvent, effectively extracts polar secondary metabolites. During extraction, diffusion occurs as ethanol penetrates plant cells, causing cell membranes to rupture and release metabolites into the solvent (Harbone, 1998; Naviglio et al., 2023). The color changes observed in the filtrates indicate successful extraction of active compounds. The filtrates were concentrated using a rotary evaporator, which separates solvent from extract by lowering pressure and temperature to evaporate ethanol without degrading the compounds (Muiz et al., 2022).

Extraction yield reflects the efficiency of the process and the amount of active compounds extracted. *K. galanga* showed the highest yield (36.7%), indicating a greater amount of extractable polar metabolites, while *C. longa* had the lowest yield (18.2%). The solubility of secondary metabolites depends on the polarity match between solute and solvent, explaining the higher yield in *K. galanga* with polar ethanol (Abubakar et al., 2020) Additionally, yield is influenced by factors such as soaking time and sample quantity (Sayuti, 2017).

The varying extraction yields were reflected in the subsequent phytochemical analysis. Phytochemical screening is used to identify active secondary metabolites in plants that may have therapeutic potential and help elucidate their biological mechanisms. (Agustina & Handayani, 2017; Vifta & Advistasari, 2018)**.** In this study, ethanol extracts of *C. zanthorrhiza*, *Z. officinale*, *C. longa*, *K. galanga*, and *jamu cekok* were screened for alkaloids, saponins, flavonoids, and tannins. Notably, *K. galanga*, which had the highest extraction yield (36.7%), tested positive only for alkaloids in this study. All samples tested positive for alkaloids and negative for tannins. Saponins were detected only in *Z. officinale* and *jamu cekok* extracts, while flavonoids were found only in *C. longa* and *jamu cekok*. The detection of only alkaloids in the high-yield *K. galanga* extract, and the limited presence of other metabolites across all samples, differed from previous reports where additional compounds such as flavonoids and terpenoids were found. This disparity is likely due to the combined effect of not optimal simplicia quality (high moisture) and the specific solvent concentration used. The absence of some metabolites, especially flavonoids and saponins, may be attributed to the use of 96% ethanol, as lower ethanol concentrations (e.g., 70%) have been shown to extract higher flavonoid content due to increased solvent polarity (Khairunnisa et al., 2022). Thus, solvent concentration, in conjunction with initial sample quality, significantly influences the extraction efficiency and the resulting profile of secondary metabolites.

Antioxidant activity of jamu cekok and its constituent

The ethanol extract of *jamu cekok* exhibited the strongest antioxidant activity with an IC_{50} value of 46.904 ppm. This finding indicates a synergistic effect among the active compounds from the four individual extracts, which enhances their capacity to scavenge free radicals (Vaou et al., 2022). The notably high antioxidant activity of jamu cekok can be attributed to the combined presence of alkaloids, flavonoids, and tannins identified in the extract. Although jamu cekok demonstrated very strong antioxidant activity, its effect was still lower than that of the positive control, quercetin.

In general, interactions between bioactive compounds can be antagonistic, additive, or synergistic. Synergistic interactions occur when the combined effect of compounds exceeds the sum of their individual effects. Such interactions may also arise when certain compounds facilitate the absorption, bioavailability, or metabolism of others, thereby enhancing therapeutic activity. The chemical complexity of medicinal plants allows such synergistic effects, which may modulate biochemical pathways, alter membrane potentials, and affect receptor selectivity. In the case of jamu cekok, the observed high antioxidant activity supports the hypothesis that the interaction among its phytochemical constituents provides a synergistic and not just an additive effect of the individual extracts (Vaou et al., 2022). Evidence from previous studies supports this interpretation. A study combining *Centella asiatica* and *C. xanthorrhiza* demonstrated much lower IC50 values for the mixture (26-42 µg/mL) compared to the individual extracts (94-96 µg/mL), indicating a clear synergistic effect (Dewi Kurnia et al., 2025). Similarly, combinations of *C. xanthorrhiza* and *Physalis angulata* produced a Combination Index (CI) < 1, confirming synergistic antioxidant activity (Setiawan et al., 2023). Furthermore, enrichment of traditional jamu beras kencur with turmeric increased total phenolic content and antioxidant capacity, underscoring the importance of multi-herbal combinations (Fitriansyah et al., 2024).

Among the individual ethanol extracts, *C. zanthorrhiza*, *Z. officinale*, and *C. longa* showed strong antioxidant activities with IC_{50} values of 96.158 ppm, 87.040 ppm, and 83.855 ppm, respectively. Strong antioxidant activity is generally classified within the range of 50–100 ppm (Marianne et al., 2018). Linear regression analysis between extract concentration and antioxidant inhibition percentage yielded correlation coefficients of 0.997, 0.995, and 0.997 for *C. zanthorrhiza*, *Z. officinale*, and *C. longa*, respectively, indicating a positive correlation where higher extract

concentrations correspond to greater antioxidant activity. This relationship reflects the higher abundace of antioxidant compounds at elevated concentrations, enabling hydrogen atoms donation to effectively inhibit free radical reactions. Differences in IC_{50} values are likely due to variation in the types and amounts of secondary metabolite groups with antioxidant properties, influenced by the solvent polarity used for extraction (Marianne et al., 2018). The highest antioxidant activity in *C. longa* may result from the combined contribution of alkaloids and flavonoids demonstrated in phytochemical screening.

In contrast, the ethanol extract of *K. galanga*, tested at concentrations of 50, 60, 80, 100, and 120 ppm, produced an IC_{50} value of 112.277 ppm, indicating moderate antioxidant activity falling within the 100–150 ppm range (Marianne et al., 2018). This moderate activity may be explained by its phytochemical profile, which revealed the presence of alkaloids only, differentiating it from the other extracts that contain a broader spectrum of antioxidant compounds.

Alkaloids act as antioxidants due to nitrogen atoms in their structure that possess free electron pairs. These free electron pairs play a role in reducing free radical activity in the body. The antioxidant mechanism of alkaloids involves donating hydrogen atoms to free radicals, thereby functioning as effective primary antioxidants by breaking free radical chain reactions, preventing the formation of new free radicals, and generates more stable products (Kartika et al., 2020).

Flavonoids, as polyphenolic compounds, contain multiple phenolic hydroxyl groups that contribute to diverse biological activities. A key property of flavonoids is their ability to complex with metal ions. Additionally, flavonoids interact with proteins, both enzymes and structural proteins, which contribute to various pharmacological effects, including enhanced connective tissue strength. As antioxidants, flavonoids neutralize free radicals through hydrogen atom donation. The interaction between flavonoids and DPPH radicals produces more stable flavonoid phenoxyl radicals. The stability of these radicals is attributed to the delocalization of unpaired electrons around their aromatic rings, which slows the propagation rate of autoxidation reactions (Wartono et al., 2021).

Although each individual extract from the herbs comprising *jamu cekok* (*C. zanthorrhiza*, *Z. officinale*, *C. longa*, *K. galanga*) demonstrated satisfactory antioxidant activity, their combination in jamu cekok exhibited a more potent synergistic effect. This is evidenced by the lower IC_{50} value in the jamu cekok extract, indicating a beneficial interaction between alkaloids and flavonoids as the main active compounds. To further confirm the therapeutic potential of jamu cekok as a natural antioxidant source, additional studies using more complex biological models, such as animal testing, are strongly recommended to elucidate its long-term effects.

CONCLUSIONS

The *jamu cekok* extract exhibited very strong antioxidant activity with an IC50 value of 46.904 ppm, exceeding those of its individual herbal constituents—temulawak, turmeric, ginger, and kencur. Extracts of temulawak, turmeric, and ginger demonstrated strong antioxidant effects, while kencur showed moderate activity. The superior antioxidant capacity of the *jamu cekok* extract is likely due to synergistic interactions among its diverse secondary metabolites. These findings support the therapeutic potential of jamu cekok as a natural antioxidant source.

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