



Antioxidant Activity of the Combination of *Morinda citrifolia* and *Alpinia galanga* in Juice and Instant Beverage Powder Using the ABTS Method

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Abstract: A combination of *Morinda citrifolia* and *Alpinia galanga* is available as a traditional herbal product in two dosage forms, namely juice and instant beverage powder, known as *kudu-laos jamu*, which potentially exhibits an antioxidant effect. The research novelty of this study lies in the comparison of the antioxidant activity between two dosage forms using the ABTS method. Six formulations were evaluated, including single-plant and combined formulations in both juice (F1–F3) and powder (F4–F6) forms. The antioxidant activity was determined quantitatively by the IC₅₀ value. The results showed that all formulations exhibited antioxidant activity; however, their IC₅₀ values were higher than that of ascorbic acid (< 50 µg/mL), indicating very weak activity (> 200 µg/mL). Statistical analysis showed a significant difference between dosage forms; the IC₅₀ value in instant beverage powder formulations was higher compared with juice formulations. This condition is attributed to dilution by sucrose as the matrix in the powder formulations and to the thermal degradation of bioactive compounds during the processing stage. Therefore, further formulation optimization is required to preserve antioxidant potential in future studies.

Keywords: ABTS; *Alpinia galanga*; Antioxidant; *Kudu-laos*; *Morinda citrifolia*

Introduction

An in vitro assay of the antioxidant activities of a combination of *Morinda citrifolia* and *Alpinia galanga* is the central focus of this study, despite numerous studies describing related bioactive compounds and their antioxidant activity. No previous studies have examined the antioxidant potential of this specific combination. A traditional beverage, manufactured by a Surabaya-based small and medium-sized enterprise, is comprised of a combination of *M. citrifolia* and *A. galanga*. It is named *kudu-laos jamu*. This SME product has been locally perceived as an immune booster beverage since the

COVID-19 pandemic. To address this gap, the study evaluates the antioxidant activities of two dosage forms (juice and powder) and compares their antioxidant properties.

Antioxidants play an essential role in inhibiting oxidation reactions by scavenging free radicals and highly reactive molecules, thereby delaying, controlling, or preventing the occurrence and progression of many chronic diseases (Gulcin, 2020; Munteanu & Apetrei, 2021; Sogandi & Rabima, 2019). Plants' high content of compounds with antioxidant properties has potentially been used in prophylactic and curative phytotherapy (Munteanu & Apetrei, 2021).

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Kudu-laos jamu is a traditional health beverage that combines *M. citrifolia*, known as *mengkudu*, and *A. galanga*, known as *laos*. The main ingredients of this product contain various kinds of secondary metabolites, which have been studied in vivo and in vitro for their antioxidant properties. A previous study reported that the IC₅₀ value of ripened fruit juice from *M. citrifolia* is 24.92 µg/ml, indicating very strong antioxidant activity under reported assay conditions (Meilawati et al., 2021). Meanwhile, the IC₅₀ value of *A. galanga* rhizome extract is 88.94 µg/ml, indicating moderate activity (Aziz et al., 2024). Moreover, extracts of these plants have demonstrated attenuation of oxidative stress in a liver disease mouse model (Wang et al., 2022) and in humans (Laksmitawati et al., 2022).

The major antioxidant constituents of *kudu-laos jamu* are polyphenol compounds. Polyphenols can be found in all parts of plants, including leaves, roots, wood, bark, pollen, nectar, flowers, fruits, and seeds (Banjarnahor & Artanti, 2014; Laksmitawati et al., 2022; Nursamsiar et al., 2025). Polyphenol constituents from plants have been predominantly extracted in ethanol, hexane, and chloroform fractions, but are rarely extracted in water fractions, especially when used in combination of both plants in juice and powder, which use water as a solvent to serve the products.

M. citrifolia has an unpleasant taste and sour smell, even though it has already been processed in the form of fruit juice (Hou et al., 2025). In addition, *A. galanga* is preferred to be used as a spice in cooking rather than ingested orally in the form of food or drink because of its strong, aromatic scents, and spicy taste (Sahoo et al., 2020). Nevertheless, due to its sensory limitations, *M. citrifolia* is consumed as a healthy beverage for the preventive and supportive management of various conditions, including stomachaches, diarrhea, diabetes, microbial infections, bronchitis, and chronic enteritis (Arjita et al., 2023; Rajivgandhi et al., 2020; Sahoo et al., 2020). Most researchers investigating *kudu-laos* have utilised it for the pharmacological activities in eliminating vaginal discharge, relieving menstrual pain in women, improving blood circulation, controlling blood sugar in diabetic patients, and reducing cholesterol (Adriani & Pritasari, 2024; Andajani et al., 2023).

Kudu-laos jamu in Surabaya has been consumed over the years, starting from its availability in juice form, which is made from a combination of squeezed *mengkudu* fruit and *laos* rhizome, since 2018, to its current transformation into a powder form combining both plants. Setiawan et al. (2025) reported that this transformation has had a positive impact on improving efficiency in the production process, enhancing product stability and shelf life, and improving packaging and

labeling design that aims to maintain product quality and attract customers.

The novelty of this study lies in the systematic evaluation of antioxidant activity from a commercially available herbal formulation, with direct comparison of dosage forms prepared using water as the extraction or dilution medium. Antioxidant activity information is important in relation to the health benefits intended to increase public trust in herbal products. The objective of this study was to evaluate the antioxidant activity of a combination of *M. citrifolia* and *A. galanga* in juice and instant beverage powder using the ABTS method

Method

Material and Instrumentation

The materials used in this research included 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate (K₂S₂O₈) and ascorbic acid, which were of analytical grade and obtained from Sigma-Aldrich. *Morinda citrifolia* commonly known as *kudu* or *mengkudu* fruit and *Alpinia galanga* also referred to as *laos* rhizome were purchased from local markets in Surabaya, East Java. They were identified and authenticated at *Pusat Informasi dan Pengembangan Obat Tradisional* (PIPOT), Universitas Surabaya, Surabaya, East Java. Sugar (Rose Brand) and distilled water were used as ingredients in the preparation of *kudu-laos jamu* in juice and instant beverage powder.

The instruments used in this research were a moisture analyzer (Mettler Toledo), a microplate reader (Biochrom UVM 340), a 96-well clear polystyrene microplate (Biologix), and a blending machine (Miyako).

Sample Preparation

M. citrifolia and *A. galanga* were the primary ingredients used in the preparation of *kudu-laos jamu* in juice and instant beverage powder. The formula was produced by following the SME's recipe (Table 1). *Mengkudu* fruit and *laos* rhizomes were thoroughly cleaned and chopped into small pieces. They were mixed with water either individually or in combination, followed by blending and filtration. The resulting filtrate was collected as the juice formulation of *kudu-laos jamu*, comprising single *M. citrifolia* or *kudu* as Formula 1 (F1), single *A. galanga* or *laos* as Formula 2 (F2), and a combination of *M. citrifolia* and *A. galanga* or *kudu-laos* as Formula 3 (F3). Additionally, sugar was added to the filtrate, either individually or in combination, followed by heating using a direct heating method to obtain the instant beverage powder. Accordingly, *single kudu* as instant beverage powder was designated as Formula 4 (F4), *single laos* as Formula 5 (F5), and *kudu-laos* as Formula 6 (F6). Formulations F1, F2, F4, and F5 were

used as comparative controls to evaluate the antioxidant effectiveness of single-plant formulations relative to the combined formulations F3 and F6.

Finished Product Quality Assay

The juice and instant beverage powder were tested for their organoleptic specification, including shape, color, smell, and flavor. Panelists recorded their observations on shape, color, odor, and taste using an assessment description sheet to identify the organoleptic specifications of both products (SNI 01-2346-2006, section 5.4).

Kudu-laos jamu, an instant beverage powder was tested for its moisture content using a moisture analyzer. Approximately 5 g of powder was placed in a moisture analyzer and heated at 105 °C for 10 minutes. Subsequently, the moisture content was determined by calculating the difference between the initial and final weights divided by the initial weight and was expressed as a percentage (SNI 01-4320-1996).

ABTS Assay

The antioxidant activity was evaluated using the ABTS radical cation (ABTS^{•+}) scavenging assay (Re et al., 19999; Yu et al., 2013). The stock solution of ABTS 7 mM was prepared in water and reacted with potassium persulfate 2.45 mM in equal volume, then incubated in the dark at room temperature for 12-16 hours. The ABTS^{•+} was then diluted with distilled water to obtain an absorbance suitable for measurement at 726 nm.

A 25 µl aliquot of diluted ABTS radical solution was added to 200 µl of each formula of *kudu-laos jamu* in juice, 1800-9000 µg/ml of F1, 900-4500 µg/ml of F2, and 1900-9500 µg/ml of F3. A 20 µl aliquot of diluted ABTS radical solution was added to 180 µl of each formula of *kudu-laos jamu* in instant beverage powder, 680-55000 µg/ml of F4, 4800-141000 µg/ml of F5, and 670-54000 µg/ml of F6. The positive control, ascorbic acid, was prepared under identical conditions, with the sample replaced by ascorbic acid substituting with the substitution of ascorbic acid for the formula at 3.50-23 µg/ml.

The absorbance was then measured at 726 nm using a microplate reader. The percentage of ABTS radical scavenging activity was calculated as:

$$\% inhibition = \frac{Ac-As}{Ac} \times 100\% \tag{1}$$

where Ac and As referred to the absorbance of the control and the sample, respectively (Nadjamuddin et al., 2023).

The half-maximal inhibitory concentration (IC₅₀) value was calculated using the regression equation $y = a + bx$, where y value = 50 and x represents the

concentration of a compound. The IC₅₀ value indicates the concentration of a compound required to inhibit 50% of free radicals (Meilawati et al., 2021).

Statistical Analysis

All experiments were performed in triplicate, and the differences were analyzed using analysis of variance (ANOVA) with SPSS version 27.0.

Result and Discussion

The *kudu-laos jamu* is a product originating from a small, home-based enterprise in Surabaya, operated by Mr. Lamiran together with his family. This bussines began by utilizing herbs around his house using culinary equipment. Producers of *jamu* or traditional remedies primarily depend on empirical knowledge and inherited formulation in their products. Formulations are generally preserved to uphold public trust in their longstanding therapeutic efficacy. Moreover, consumer acceptance of *jamu* is heavily influenced. The organoleptic properties, particularly bitterness and acidity, are inherent due to plant-derived bioactive compounds. *Jamu* is rarely served in concentrated extract, but is generally served as a diluted solution. To enhance acceptability, one strategy is to incorporate natural sweeteners, such as sucrose. Transforming *kudu-laos jamu* from juice into a powder form serves to enhance consumer acceptance (Setiawan et al., 2025). Yusransyah et al. (2023) reported that this transformation renders the products more durable, more portable, and versatile for consumption either hot or cold.

Table 1. *Kudu-laos jamu* formulation

Ingredients	Juice formula			Instant beverage powder formula		
	F1	F2	F3	F4	F5	F6
<i>Morinda citrifolia</i> (g)	150	-	150	150	-	150
<i>Alpinia galanga</i> (g)	-	3	3	-	3	3
Water (ml)	ad	ad	ad	ad	ad	ad
Sugar (g)	300	300	300	300	300	300
	-	-	-	300	300	300

In the study, *M. citrifolia* and *A. galanga* were processed by washing them with running water to clean dirt that adhered to the plants. Meanwhile, the chopping stage was carried out to reduce the size so that the next step, the blending and filtering stage, was faster and made it easier to obtain the filtrate (Nursamsiar et al., 2024). F1, F2, and F3 were the results of the collected filtrate to produce *kudu-laos jamu* in juice. Adding sugar to the filtrate by heating and constantly stirring is the

crystallization step to produce F4, F5, and F6 of *kudu-laos jamu* in instant beverage powder.

The cocrystallization method is a method that uses sucrose as a coating material or a crystallization agent. In this study, the addition of sugar also acted as a sweetener and preservative. This method involves heating and continuous stirring to release heat into the system until caramelization occurs. Heat is still required to dry the crystals, resulting in a separate dry powder (Fortin et al., 2021). Heating at high temperatures and stirring too slowly can cause the powder to burn and taste bitter. In contrast, heating at low temperatures will take a long time to crystallize and cause clumping (Yuliastuti, 2022).

Table 2. *Kudu-laos jamu* organoleptic tests

Formula	Parameters			
	Shape	Color	Smell	Flavor
F1	Solution	Brownish yellow	Sour	Bitter
F2	Solution	Pale yellow	Strong, aromatic	Spicy
F3	Solution	Brownish yellow	Sour	Bitter
F4	Powder	Brownish yellow	Slightly sour	Sweet
F5	Powder	Pale yellow	Slightly strong, aromatic	Sweet
F6	Powder	Brownish yellow	Slightly sour	Sweet

An organoleptic evaluation was performed to assess the smell, color, taste, and texture of *kudu-laos jamu* in juice and instant beverage powder (Table 2). *Kudu-laos jamu* in juice (F1, F2, and F3) resulting from the formulation process had a solution form, whereas instant beverage powder (F4, F5, and F6) had a powder form. The color of F1, F3, F4, and F6 is brownish yellow because they come from *M. citrifolia*, which was more widely used in the formulation than the others, while F2 and F5 is pale yellow because they comes from *A. galanga*. There is no addition of dye, so the color produced in this formulation is original. The smell of F4 and F6 is slightly sour compared to F1 and F3 because they are coated with sugar, similarly to F2, which has strong, aromatic rhizome notes compared to F5. The flavor of F1 and F3 is bitter compared to F2, which is spicy. Meanwhile, F4, F5, and F6 are sweet.

The differences in organoleptic results may have occurred due to differences in the chemical and physical properties of the two ingredients as well as their interaction during the formulation process. The medium-chain fatty acids, such as hexanoic acid (caproic acid), octanoic acid (caprylic acid), and decanoic acid (capric acid) in *M. citrifolia* release a strong rancid-like odor, whereas *A. galanga* has various volatile

compounds like camphene, 1,8-cineole, camphor, and others, which release aromatic and spicy flavors (Laksmiawati et al., 2022; Sahoo et al., 2020; Yien et al., 2016). Differences in the concentration of ingredients within the formulation can affect organoleptic results; if *M. citrifolia* is used more than *A. galanga*, the resulting product tends to exhibit dominant sour and bitter characteristics (Kamadia et al., 2006). Meanwhile, the addition of sugar, matrix composition, and processing methods in the formulation can affect the release of volatile compounds (Sardar & Singhal, 2013). Sugar can mask the unpleasant taste; consequently, the powder form is better suited for *M. citrifolia* to be retained within the matrix, while heating in juice form intensifies the undesirable aromas, leading to distinct organoleptic characteristics. In addition, the powder form aims to simplify preparation because it dissolves quickly in hot or cold water and there is no need to add sugar separately, extends shelf life, and facilitates packaging and distribution (Rahmawati et al., 2024; Ulfah et al., 2023).

Kudu-laos jamu in instant beverage powder (F4, F5, and F6) was then analyzed for moisture content (Table 3). This test is required to ensure product quality by resisting microbial growth and maintaining stability during storage (Mawardi et al., 2016). The moisture content of F4 and F6 (4.13 and 4.77%, respectively) was higher than that of F5 (0.83%). Compared with the maximum requirement of 3% specified in SNI 01-4320-1996, these results did not meet the standard. This was caused by the use of *M. citrifolia* in F4 and F6 which has a high water content compared to F5, which only used *A. galanga* (Arfa & Ghannam, 2022; Samarasinghe et al., 2023). In addition, the high-water content in cocrystallized powder is the result of sugar's hygroscopic properties and the crystalline matrix formed during cocrystallization, which traps water or hydration water. This phenomenon is difficult to remove during heating, preventing the powder from reaching low moisture levels below 3% (Farshchi et al., 2025; Schugmann & Foerst, 2024).

Table 3. *Kudu-laos jamu* moisture content tests

Formulation	Water content (%)
F4	4.13 ± 0.03
F5	0.83 ± 0.04
F6	4.77 ± 0.07

The antioxidant activity of *kudu-laos jamu*, a combination of *M. citrifolia* and *A. galanga*, in juice and instant beverage powder was evaluated based on the decolorization of the ABTS cation radical, which changes from deep bluish-green to colorless after reacting with antioxidant compounds (Ilyasov et al., 2020; Meilawati

et al., 2021). The ABTS+ was generated by oxidizing ABTS with K₂S₂O₈, followed by incubation for 12-16 hours under dark conditions due to its light sensitivity (Meilawati et al., 2021; Nursamsiar et al., 2024). Based on Figure 1, ascorbic acid exhibited a very strong level of antioxidant activity with an IC₅₀ value of 10.90 µg/ml (< 50 µg/ml, whereas the *kudu-laos jamu* fractions (F1-F6) showed IC₅₀ values higher than ascorbic acid (> 200 µg/ml), indicating very weak antioxidant activity (Dewi & Sari, 2024). A lower IC₅₀ value indicates stronger antioxidant activity, as a lower concentration is required to scavenge free radicals (Kurniati et al., 2019).

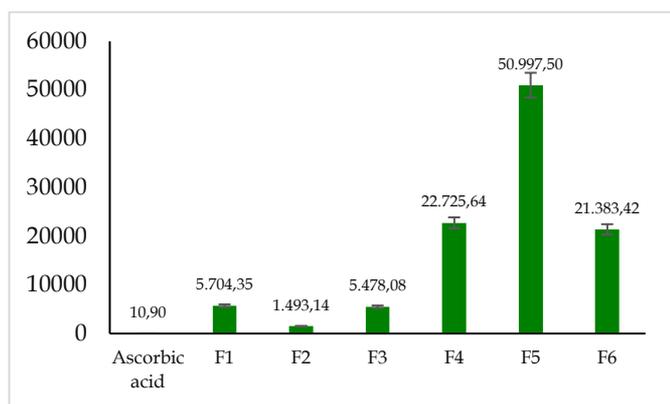


Figure 1. Graph of antioxidant activity of *kudu-laos jamu* using the ABTS method

Table 4. Antioxidant activities of *kudu-laos jamu*

Formulation	IC ₅₀ (µg/ml)
Ascorbic acid	10.90 ± 0.07
F1	5704.35 ± 116.08*
F2	1493.14 ± 36.98**
F3	5478.08 ± 74.30*
F4	22725.64 ± 68.12*
F5	50997.50 ± 734.04**
F6	21383.42 ± 240.54*

*P < 0.01, ** P < 0.05, significant when compared each formula

The statistical analysis results of the antioxidant activity of *kudu-laos jamu* in juice and instant beverage powder are presented in Table 4. A significant difference in antioxidant activity was observed in instant beverage powder formulations (F4-F6), which exhibited higher IC₅₀ values compared to juice formulations (F1-F3). This increase is attributed to the dilution effect associated with the matrix composition of the instant beverage powder. It contains a substantial amount of sucrose, thereby increasing the overall mass of the test material. Consequently, since IC₅₀ is expressed in µg/ml of the final product powder, a higher concentration is required to achieve 50% radical inhibition, resulting in an upward shift. This reduction in activity may be attributed to the temperature and duration of heating during the

production process. Both factors can lead to the decomposition of bioactive compounds, resulting in a decrease in antioxidant activity (Kurniati et al., 2019; Permanasari et al., 2021). Kurniati et al. (2019) reported that high temperatures and prolonged heating during processing can significantly reduce the total phenolic and flavonoid levels in kudu fruit, consequently decreasing its antioxidant activity. Furthermore, the addition of sugar can also diminish the antioxidant activity, as sugar acts as a hydrogen donor to free radicals due to the presence of methyl groups and hydrogen atoms (Permanasari et al., 2021).

Conclusion

The combination of *Morinda citrifolia* and *Alpinia galanga* in juice and instant beverage powder exhibits antioxidant activity, although markedly lower than ascorbic acid, with IC₅₀ values classified as very weak. A significant difference was observed between dosage forms; IC₅₀ values in instant beverage powder were higher than those of juice formulations, indicating reduced antioxidant effectiveness. Therefore, future research should focus on optimizing processing conditions to reduce thermal degradation and improve antioxidant activity, optimizing the matrix formulation to reduce the sucrose content or use alternative carriers, and conducting phytochemical profiling studies to validate the biological relevance of this combination formulation.

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Author Contributions

Conceptualization V.S, C.H.A.M, A.H.K. Investigation C.A.A, V.S, C.H.A.M, A.H.K, R.B. Data curation C.A.A. Drafting C.H.A.M, V.S. Review and Editing V.S, C.H.A.M.

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Conflicts of Interest

The authors declare no conflicts of interest.

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