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Effect of Red Galangal–Citronella Grass Decoction Ratios on Phenolic Content, Flavonoid Content, and Antioxidant Activity

Pengaruh Variasi Rasio Dekokta Lengkuas Merah–Serai Wangi terhadap Kadar Fenolik, Kadar Flavonoid, dan Aktivitas Antioksidan

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ABSTRACT

Whether the plants in polyherbal decoctions interact is rarely tested. Red galangal (*Alpinia purpurata*) and citronella grass (*Cymbopogon nardus*) were decocted at ratios of 100:0, 75:25, 50:50, 25:75, and 0:100 (w/w) and analysed for total phenolic content (TPC), total flavonoid content (TFC), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging activity; mixtures were compared with additive expectations. TPC decreased from 593.84 to 375.75 mg gallic acid equivalents/L as citronella grass increased, whereas TFC peaked at 25:75 (49.90 mg quercetin equivalents/L). The half-maximal inhibitory concentration (IC_{50}) ranged from 1106.64 µg/mL (red galangal) to 1682.97 µg/mL (citronella grass). Every mixture's activity matched the additive prediction (observed/expected 0.96–1.05, $p > 0.3$), whereas TFC deviated strongly (0.74–1.41). Activity correlated with TPC ($r = 0.97$, $p = 0.007$) but not TFC. The ratio therefore adjusts antioxidant capacity proportionally, without synergy, and phenolics rather than flavonoids govern it.

Kata kunci:

ABTS
Alpinia purpurata
Cymbopogon nardus
Dekokta

ABSTRAK

Interaksi antartanaman penyusun dekokta poliherbal jarang diuji. Lengkuas merah (*Alpinia purpurata*) dan serai wangi (*Cymbopogon nardus*) didekoksi pada rasio 100:0, 75:25, 50:50, 25:75, dan 0:100 (b/b), lalu diukur kadar fenolik total (TPC), kadar flavonoid total (TFC), dan aktivitas penangkapan radikal asam 2,2′-azino-bis(3-etilbenzotiazolin-6-sulfonat) (ABTS); nilai campuran dibandingkan dengan nilai harapan model aditif. TPC menurun dari 593,84 menjadi 375,75 mg ekuivalen asam galat/L seiring bertambahnya serai wangi, sedangkan TFC tertinggi pada rasio 25:75 (49,90 mg ekuivalen kuersetin/L). Konsentrasi penghambatan 50% (IC_{50}) berkisar 1106,64 µg/mL (lengkuas merah) hingga 1682,97 µg/mL (serai wangi). Aktivitas setiap campuran sesuai prediksi aditif (rasio observasi/harapan 0,96–1,05; $p > 0,3$), sedangkan TFC menyimpang kuat (0,74–1,41). Aktivitas berkorelasi dengan TPC ($r = 0,97$; $p = 0,007$), tetapi tidak dengan TFC. Rasio formulasi mengatur kapasitas antioksidan secara proporsional tanpa sinergi, dan fenolik, bukan flavonoid, yang menentukannya.

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1. INTRODUCTION

Oxidative stress arises when the production of reactive oxygen and nitrogen species exceeds the capacity of the body's antioxidant defences, and it is a common denominator of many chronic and degenerative diseases (Jomova et al., 2023). Dietary polyphenols from plants can limit this damage by scavenging reactive species before they react with lipids, proteins, and DNA (Rudrapal et al., 2022). In Indonesia such antioxidants are most often consumed as decoctions, the main mode of preparation of traditional remedies (Elfrida et al., 2021), and traditional formulas usually combine several plant materials in one preparation (Widyowati et al., 2024). Whether the plants in such a formula act independently, or enhance or suppress each other's activity, is rarely tested (Wardani et al., 2025; Yap et al., 2023).

Phenolic and flavonoid compounds are the secondary metabolites most often associated with the antioxidant activity of herbal infusions and decoctions (Fotakis et al., 2016). Red galangal (*Alpinia purpurata* (Vieill.) K.Schum.) is known to contain phenolic compounds and several other bioactive constituents with antioxidant potential (Mardhiyyah et al., 2021); constituents such as cinnamic acid, 4-coumaric acid, homovanillic acid, and shogaol have been tentatively identified in an ethanolic extract of the rhizome (Mulkiya Yuliawati et al., 2026), whereas data on aqueous preparations of this species remain limited. Meanwhile, citronella grass (*Cymbopogon nardus* (L.) Rendle) contains flavonoids and several phytochemical compounds that contribute to free radical scavenging activity (Chairina et al., 2023); phenolics, tannins, alkaloids, and flavonoids have also been detected in its ethanolic extract (Rohmawaty et al., 2025), whereas information on the metabolites recovered in water is limited; in the closely related lemongrass (*C. citratus*), decoctions are dominated by water-soluble phenolic acids such as caffeic and syringic acids (Muala et al., 2021). Because water preferentially extracts polar constituents, the composition of a decoction cannot be inferred directly from data obtained using organic solvents. Decoction is one of the extraction methods commonly applied in traditional herbal preparation because it is simple, economical, and eco-friendly (Hasan et al., 2022). This method uses water as a solvent, making it suitable for extracting polar compounds such as phenolics and flavonoids (Harouak et al., 2021).

Previous studies have mostly focused on the antioxidant activity of single plant extracts, while combined herbal formulations, particularly decoctions prepared at different ratios, remain little studied (Chairina et al., 2023). In a mixture, constituents can interact so that the measured activity is higher (synergy), lower (antagonism), or equal (additivity) to the sum of the contributions of the individual components (Olszowy et al., 2019; Yap et al., 2023), and the outcome depends on phytochemical composition, formulation ratio, and extraction method (De Marino et al., 2014; Rama et al., 2023). Distinguishing these cases requires comparing each mixture with the value expected from its components, rather than ranking mixtures by their raw values (Rahim et al., 2020; Uduwana et al., 2023). For red galangal and citronella grass this comparison has not been made, so it is not known whether combining them in a decoction does more than dilute one plant with the other.

The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay was chosen because its radical cation is soluble in both aqueous and organic media, so that it detects hydrophilic and lipophilic antioxidants, absorbs in the visible region, and reacts rapidly (Anwar et al., 2022). This study aimed to determine how the ratio of red galangal rhizome to citronella grass stem in a water decoction affects total phenolic content (TPC), total flavonoid content (TFC), and ABTS radical-scavenging activity, and to test whether the mixtures behave additively or show synergy or antagonism. If the two plant materials do not interact, each property of a mixture should equal the mass-weighted mean of the two single-plant decoctions.

2. METHODS

2.1. Instruments and Materials

Spectrophotometric analysis was carried out using an ultraviolet–visible (UV–Vis) spectrophotometer Cary 60 UV–Vis (Agilent Technologies, California, USA). Other equipment used in this study included a laboratory-made drying oven (manufacturer unavailable), moisture analyzer MOC63U UniBloc (Shimadzu Corporation, Kyoto, Japan), analytical balance (Mettler Toledo, Greifensee, Switzerland), blender (Getra, Zhejiang, China), 40-mesh sieve, and standard laboratory glassware (Iwaki, AGC Group, West Java, Indonesia).

The plant materials used in this study were red galangal rhizome (*A. purpurata*) collected from Pasekan Village, Ambarawa District, Semarang Regency, Central Java, Indonesia, and citronella grass stem (*C. nardus*) collected from Rejosari Village, Jambu District, Semarang Regency, Central Java, Indonesia. Botanical authentication of plant materials was conducted at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Islam Sultan Agung, Indonesia, based on taxonomic characteristics. The identification reference numbers were No. 027/B.1./SA-F/X/2025 for *A. purpurata* and No. 026/B.1./SA-F/X/2025 for *C. nardus*.

The reagents used in this study included ABTS (analytical grade, Sigma-Aldrich, Steinheim, Germany), potassium persulfate (analytical grade, EMSURE, Darmstadt, Germany), ethanol p.a. (≥99.8%, Merck, Darmstadt, Germany), aluminum chloride ($AlCl_3$, analytical grade, Merck, Darmstadt, Germany), sodium acetate (analytical grade, EMSURE, Darmstadt, Germany), sodium carbonate (Na_2CO_3, analytical grade, EMSURE, Darmstadt, Germany), Folin–Ciocalteu reagent (Merck, Darmstadt, Germany), magnesium powder (analytical grade, Merck, Darmstadt, Germany), concentrated hydrochloric acid (37%, Merck, Darmstadt, Germany), ferric chloride ($FeCl_3$, analytical grade, Merck, Darmstadt, Germany), gallic acid (≥98%, Sigma-Aldrich, Steinheim, Germany), quercetin (≥95%, Sigma-Aldrich, Steinheim, Germany), vitamin C (analytical grade, Merck,

Darmstadt, Germany), and distilled water (Ikapharmindo Putramas, Jakarta, Indonesia).

2.2. Sample Preparation and Decoction Extraction

Fresh plant materials were washed thoroughly, cut into small pieces, and dried in a drying oven at 50–60 °C until constant weight was achieved. The dried samples were blended into powder and sieved through a 40-mesh sieve to obtain uniform particle size. Drying was continued until the moisture content, measured at 105 °C with a moisture analyzer, was below 10%. The powdered samples were stored in airtight dark containers at room temperature until further analysis. The ratios were prepared based on the dry weight (w/w) of red galangal and citronella grass, with a total mass of 25 g for each formulation, namely 100:0, 75:25, 50:50, 25:75, and 0:100. Extraction was performed by the decoction method using distilled water with a material-to-solvent ratio of 1:20 (w/v). The mixtures were heated at 90 °C for 30 min with occasional stirring. After heating, the mixtures were cooled to room temperature and filtered using Whatman No. 1 filter paper to obtain the decoction extracts. The fresh filtrates were used directly for the phytochemical and antioxidant analyses. This decoction procedure was adapted from a five-component crude drug decoction protocol, modified in the present study for a two-component ratio comparison, a lower heating temperature, and direct (rather than indirect, water-bath) heating (Wardani et al., 2025).

2.3. Phytochemical Screening

Phytochemical screening was performed according to Harborne (2006) to identify flavonoid and phenolic compounds in the extracts. Flavonoids were tested using magnesium powder and concentrated hydrochloric acid, which produced a red to orange coloration as a positive result. Phenolic compounds were identified using 3% ferric chloride ($FeCl_3$), indicated by the appearance of green, blue, purple, or black coloration.

2.4. Determination of Total Phenolic Content (TPC)

Total phenolic content was analyzed using the Folin–Ciocalteu method with gallic acid as the standard reference compound. The calibration curve of gallic acid showed good linearity within the concentration range of 30–70 µg/mL with the regression equation $y = 0.0073x + 0.0089$ and $R^2 = 0.9964$. Prior to analysis, the decoction extract was diluted 10-fold using distilled water (1:10, v/v). For the assay, 1 mL of the diluted sample or standard solution was mixed with 4.5 mL of diluted Folin–Ciocalteu reagent (1:10) and 4.5 mL of 7.5% sodium carbonate solution. The mixture was incubated in dark conditions for 26 min. Absorbance was recorded at 732 nm, which had been determined previously as the maximum wavelength. Distilled water mixed with the diluted Folin–Ciocalteu reagent and 7.5% Na_2CO_3 was used as the blank solution. All measurements were conducted in triplicate. Total phenolic content was calculated from the gallic acid standard curve and expressed as mg gallic acid equivalent per liter of extract (mg GAE/L extract).

2.5. Determination of Total Flavonoid Content (TFC)

Total flavonoid content was measured using the aluminum chloride colorimetric method with quercetin as the standard. Quercetin standard solutions were prepared at concentrations ranging from 30 to 70 µg/mL. The quercetin calibration curve yielded the regression equation $y = 0.0097x - 0.0133$ with $R^2 = 0.9945$. A volume of 0.5 mL of sample or standard solution was mixed with 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. The reaction mixture was incubated in dark conditions for 26 min. The absorbance was measured at 432 nm as the selected maximum wavelength. Distilled water mixed with 10% $AlCl_3$ and 1 M sodium acetate was used as the blank solution. All measurements were conducted in triplicate. Total flavonoid content was determined from the quercetin calibration curve and expressed as mg quercetin equivalent per liter of extract (mg QE/L extract).

2.6. ABTS Radical Scavenging Assay

ABTS radical cation solution was prepared by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate solution in equal volumes and incubated in dark conditions for 12–16 h at room temperature. Prior to analysis, the ABTS radical solution was diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at the selected maximum wavelength. The maximum wavelength was determined within the range of 600–800 nm, while the operating time was established based on absorbance stability.

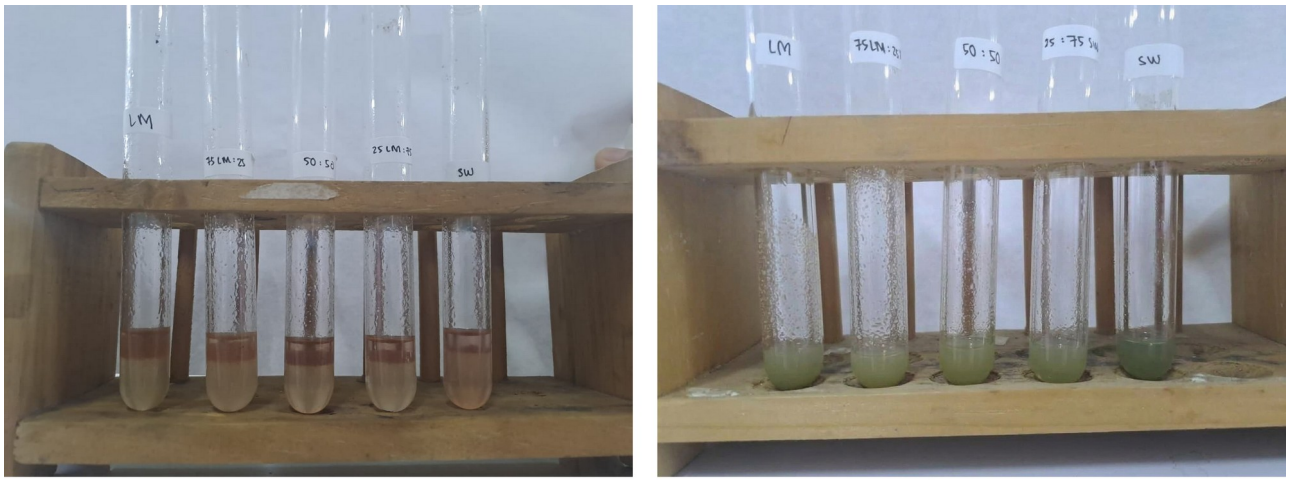
Sample solutions were prepared by diluting each decoction (25 g of dried plant material in 500 mL of distilled water, equivalent to 50 mg/mL of plant material) with distilled water to 1000–5000 µg/mL, so that sample concentrations and half-maximal inhibitory concentration (IC_{50}) values are expressed as plant-material equivalents. Vitamin C solutions were prepared at 5–25 µg/mL as the positive control. For the assay, 0.1 mL of sample or standard solution was mixed with 2 mL of ABTS solution and 1 mL of ethanol. After incubation in dark conditions for 5 min, the absorbance was measured at 728 nm using a UV–Vis spectrophotometer. All measurements were conducted in triplicate. Antioxidant activity was expressed as percentage inhibition using the following equation:

$$\text{Inhibition (\%)} = (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100 \qquad (1)$$

where A_{control} is the absorbance of the ABTS working solution without sample and A_{sample} is the absorbance with sample or standard. IC_{50} values were calculated from the linear regression equation for each sample obtained by plotting sample concentration against percentage inhibition.

2.7. Statistical Analysis

Each formulation was prepared as three independent decoctions, each extracted and analysed separately, and data are presented as mean ± standard deviation (SD). Statistical analysis was performed using Minitab Statistical Software version 22 (Minitab LLC, State College, PA, USA). Normality of the residuals of a one-way analysis of variance (ANOVA) was checked with the Shapiro–



(A) (B)

Figure 1. Phytochemical screening reactions of the red galangal–citronella grass decoctions; tubes from left to right: 100:0, 75:25, 50:50, 25:75, and 0:100 (w/w). (A) Flavonoid test with magnesium powder and concentrated hydrochloric acid; (B) phenolic test with 3% ferric chloride ($FeCl_3$). A red to orange colour indicates a positive flavonoid reaction, and a green, blue, purple, or black colour indicates a positive phenolic reaction (Mukarromah et al., 2024).

Wilk test and homogeneity of variance with Levene's test. Both assumptions were met for TFC, TPC (Shapiro–Wilk $p = 0.478$; Levene $p = 0.558$), and IC_{50} ($p = 0.617$ and $p = 0.598$), with $p > 0.05$ in every case, so differences among formulations were tested by one-way ANOVA followed by Tukey's honestly significant difference (HSD) test.

Interaction between the two plant materials was assessed with an additive model (Hossain et al., 2023; Uduwana et al., 2023). For a mixture containing a mass fraction f of red galangal, the value expected in the absence of interaction is $E = fX_{RG} + (1 - f)\cdot X_{CG}$, where X_{RG} and X_{CG} are the means of the single-plant decoctions; the SD of E was propagated from the SDs of the two components. Because IC_{50} is inversely related to activity, the model was applied to radical-scavenging activity expressed as $1/IC_{50}$, and the result was converted back to an expected IC_{50}. Each observed mean was compared with its expected value by Welch's t-test ($n = 3$), and the observed-to-expected ratio (O/E) is reported: $O/E > 1$ with $p < 0.05$ indicates a positive deviation (synergy, for activity), $O/E < 1$ with $p < 0.05$ a negative deviation (antagonism), and $p \geq 0.05$ additivity. The association of TPC and TFC with activity ($1/IC_{50}$) across the five formulations was assessed by Pearson's correlation ($n = 5$). Differences were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening

Qualitative screening detected flavonoids (red to orange colour with magnesium powder and concentrated hydrochloric acid) and phenolic compounds (green to black colour with $FeCl_3$) in all five decoctions, from red galangal alone (100:0) to citronella grass alone (0:100) (**Figure 1**). The positive reactions in both single-plant decoctions show that each material contributes both compound classes to the mixtures, which justified quantifying them in every formulation.

The consistent detection of flavonoids and phenolics across all formulations suggests that both plant materials contribute similar classes of secondary metabolites within the extract system. Similar findings have been reported in previous studies, where red galangal was shown to contain flavonoids and phenolic compounds (Mardhiyyah et al., 2021) while citronella grass contains various phenolic and flavonoid constituents such as luteolin, quercetin, and kaempferol (Tibenda et al., 2022). These results provide a qualitative basis for subsequent quantitative evaluation.

3.2. Total Flavonoid Content (TFC)

The total flavonoid content varied across all samples, indicating that the proportion of red galangal and citronella grass influenced flavonoid levels in the decoction system (**Figure 2A**). The highest value was observed in the 25:75 combination, followed by citronella grass alone, whereas the lowest content was found in the 75:25 mixture. Red galangal (28.90 mg QE/L extract) ranked third among the five formulations, higher than the 50:50 (24.92 mg QE/L extract) and 75:25 (23.12 mg QE/L extract) combinations but lower than the 25:75 combination and citronella grass alone. Tukey HSD post-hoc analysis indicated that red galangal did not differ significantly from the 50:50 combination, and that the 50:50 and 75:25 combinations were themselves statistically comparable. This pattern indicates that increasing the proportion of citronella grass contributed more prominently to the flavonoid composition, although the effect was not strictly proportional across all ratios. One-way ANOVA confirmed significant differences among formulations ($F(4,10) = 102.36$, $p < 0.0001$).

This observation is consistent with previous reports describing citronella grass as a rich source of flavonoids, including luteolin,

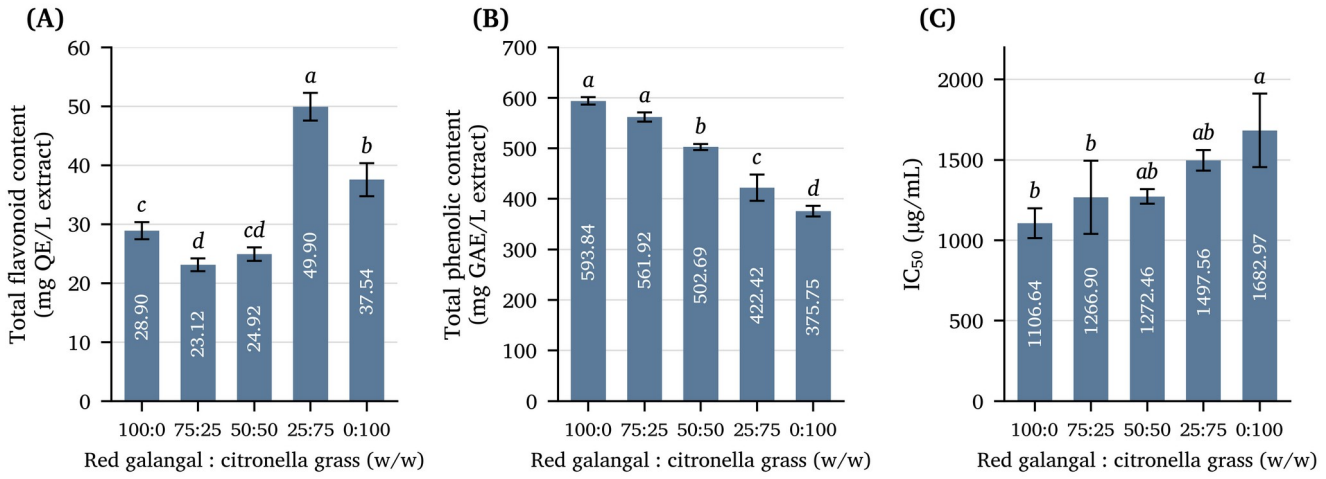


Figure 2. Total flavonoid content (A), total phenolic content (B), and ABTS radical-scavenging activity expressed as IC_{50} (C) of red galangal–citronella grass decoctions at five ratios (w/w). Bars show mean ± SD (n = 3), with the mean printed inside each bar. Different lowercase letters within a panel indicate significant differences (one-way ANOVA followed by Tukey's HSD test, $p < 0.05$): (A) $F(4,10) = 102.36$; (B) $F(4,10) = 132.48$; (C) $F(4,10) = 6.46$. Vitamin C, the positive control in (C), gave an IC_{50} of 17.64 ± 1.30 µg/mL and was not included in the statistical analysis.

quercetin, and kaempferol derivatives (Tibenda et al., 2022). Although red galangal also contains flavonoid compounds, its contribution appears less pronounced under the extraction conditions used in this study. In addition to compositional differences, the extraction process likely influences flavonoid recovery, since thermal extraction enhances flavonoid release while simultaneously affecting compound stability (Akowuah & Zhari, 2010). The $AlCl_3$ method also responds mainly to flavonols and flavones able to chelate aluminium, and its result depends strongly on the reference standard, so TFC values are better treated as relative than as absolute measures (Shraim et al., 2021).

3.3. Total Phenolic Content

The total phenolic content varied among the tested formulations, reflecting the influence of plant composition on phenolic extraction (**Figure 2B**). The highest phenolic content was observed in red galangal, followed by the 75:25 and 50:50 combinations, whereas citronella grass exhibited the lowest value. This pattern suggests that red galangal contributes more substantially to the phenolic composition of the decoction system.

The relatively high phenolic levels observed in the mixed formulations are likely attributable to the combined presence of flavonoid and non-flavonoid phenolics, which collectively contribute to the total phenolic content. In addition, the decoction process likely facilitates the extraction of water-soluble phenolics (Rauf et al., 2018). However, variations in plant matrix characteristics, such as cell wall structure and compound binding, may influence the release efficiency of these compounds across formulations (Predescu et al., 2016).

One-way ANOVA confirmed a significant overall difference among samples ($F(4,10) = 132.48$, $p < 0.0001$). Red galangal and the 75:25 combination did not differ significantly (Tukey HSD), whereas the 50:50 and 25:75 combinations and citronella grass each differed from red galangal and from one another, indicating a graded decline in phenolic content as the proportion of citronella grass increased.

When converted to the same basis (mg per gram of dried plant material, accounting for the 1:20 material-to-solvent ratio used), the phenolic content of the present decoctions corresponded to approximately 11.88 mg GAE/g dried material for red galangal and 7.52 mg GAE/g dried material for citronella grass, which is lower than the total phenolic content reported for a young coconut husk decoction prepared under comparable conditions (46.43 mg GAE/g husk powder; Salim et al., 2025). On the same basis, TFC ranged from 0.46 to 1.00 mg QE/g dried material. The total flavonoid content of the present study, however, was quantified against a different reference standard (quercetin equivalent) than that used for coconut husk decoction (rutin equivalent; Salim et al., 2025), which limits direct numerical comparison of flavonoid values between the two studies. Because the Folin–Ciocalteu reagent reacts with any reducing substance, TPC is best read as the total reducing capacity of the decoction expressed in gallic acid equivalents (Platzer et al., 2021); this does not affect the comparison between formulations, which were all analysed under the same conditions.

3.4. Antioxidant Activity

The antioxidant activity of red galangal, citronella grass, and their combinations was evaluated using the ABTS assay (**Figure 2C**). Red galangal demonstrated the strongest radical scavenging activity, indicated by the lowest IC_{50} value, while citronella grass showed the weakest antioxidant activity. The mixed formulations showed intermediate activity, with antioxidant activity generally decreasing as the proportion of citronella grass increased. In contrast, vitamin C, used as the positive control, exhibited very strong antioxidant activity (IC_{50} = 17.64 ± 1.30 µg/mL).

Following the classification of Molyneux (2004), all five decoctions (IC_{50} 1106–1683 µg/mL) fell into the very weak category (IC_{50} > 200 µg/mL), and vitamin C into the very strong category (IC_{50} < 50 µg/mL).

The IC_{50} values obtained in this study were higher than those reported for ethanolic extracts of the same plants. Wahyuni et al. (2024) reported moderate ABTS activity for an ethanolic extract of citronella grass, and an ethanolic extract of *A. purpurata* prepared by microwave-assisted extraction gave a 2,2-diphenyl-1-picrylhydrazyl (DPPH) IC_{50} of 219.06 µg/mL (Djati et al., 2025). The lower activity of the present decoctions is likely related to the use of water, which extracts mainly polar compounds and is less effective for semi-polar and non-polar antioxidants (Cui & Zhao, 2020); decoctions also generally recover fewer phenolics than infusions of the same plants (Fotakis et al., 2016). In addition, prolonged heating during decoction could reduce the stability of thermolabile compounds (Harouak et al., 2021).

Because radical scavenging in the ABTS assay is concentration-dependent, IC_{50} expresses potency per unit concentration rather than the maximum inhibition a sample can reach, and a preparation of low potency can still give high inhibition when tested at a higher concentration (Munteanu & Apetrei, 2021). The IC_{50} values reported here are therefore best read as relative potencies of the five formulations on a plant-material-equivalent basis, not as a measure of the activity of the decoctions as consumed.

Across the five formulations, radical-scavenging activity ($1/IC_{50}$) was strongly and positively correlated with TPC ($r = 0.97$, $p = 0.007$, $n = 5$) but not with TFC ($r = -0.64$, $p = 0.24$). Red galangal, which contained the most phenolics, was also the most active, and activity fell in step with TPC as citronella grass replaced it (**Figure 2B** and **Figure 2C**). A close relationship between TPC and ABTS activity is typical of aqueous herbal preparations, in which phenolic compounds account for most of the radical-scavenging capacity (Fotakis et al., 2016). Because the Folin–Ciocalteu assay measures reducing capacity rather than phenolics alone (Platzer et al., 2021), the correlation points to the reducing constituents of the decoction, of which phenolics are the largest group, as the main contributors to activity.

Conversely, formulations with higher total flavonoid content did not always demonstrate stronger antioxidant activity. This suggests that antioxidant capacity was influenced not only by flavonoid concentration, but also by the structural characteristics of the compounds present in the extract. The antioxidant behavior of flavonoids is closely related to the arrangement of hydroxyl groups and conjugated structures within the molecule, which affect their ability to donate electrons or hydrogen atoms and stabilize free radicals (Hirose et al., 2005; Procházková et al., 2011).

One-way ANOVA confirmed a significant overall difference in IC_{50} among formulations ($F(4,10) = 6.46$, $p = 0.008$). Only red galangal and the 75:25 combination differed significantly from citronella grass (Tukey HSD, $p < 0.05$); the remaining pairwise comparisons, including those among the mixtures themselves, were not significant. Activity therefore changed gradually across the series rather than in discrete steps.

3.5. Interaction Between Red Galangal and Citronella Grass

Table 1 compares each mixture with the value expected if red galangal and citronella grass contributed independently in proportion to their mass. Radical-scavenging activity was additive at every ratio: the observed IC_{50} of each mixture lay within 5% of its expected value (O/E 0.96–1.05, $p \geq 0.38$). The decoctions therefore showed neither synergy nor antagonism in the ABTS assay, and the activity of any blend can be predicted from the two single-plant decoctions. This agrees with reports that co-extracted or blended plant materials often behave additively in electron-transfer assays (Hossain et al., 2023; Kurin et al., 2025), whereas synergy in polyherbal formulations tends to appear in hydrogen-atom-transfer assays such as the oxygen radical absorbance capacity (ORAC) assay rather than in ABTS (Yap et al., 2023). Apparent antagonism in the ABTS assay can also arise from differences in reaction kinetics among antioxidants rather than from chemical interaction (Olszowy et al., 2019), but no such deviation was detected here.

Total phenolic content was slightly above the additive prediction in the 75:25 and 50:50 mixtures (by about 4%, $p < 0.05$) and additive at 25:75. A deviation of this size did not translate into any measurable change in activity and is small in practical terms. Total flavonoid content, in contrast, departed strongly from additivity: it was about 25% below the expected value in the 75:25 and 50:50 mixtures and 41% above it in the 25:75 mixture ($p < 0.01$ in each case). Opposite deviations of this size within one binary series are difficult to explain by simple dilution. Possible causes are a change in the extraction of citronella flavonoids in the presence of the red galangal matrix, complexation of flavonoids with other co-extracted constituents, or interference in the $AlCl_3$ reaction, whose response depends on flavonoid structure and on the other compounds present (Shraim et al., 2021). Opposite deviations for TFC and for TPC or antioxidant capacity within the same mixtures have also been reported for green tea infusions combined with honey and lemon extract (Uduwana et al., 2023), and a polyherbal decoction containing lemongrass showed synergistic or antagonistic interactions depending on the ratio (Rahim et al., 2020). Because activity remained additive, the TFC deviations did not affect the antioxidant capacity of the decoctions, which supports the conclusion that the flavonoids measured by $AlCl_3$ are not the main contributors to ABTS activity in this system.

These results point to several next steps. A second assay based on a different mechanism, such as the ferric reducing antioxidant power (FRAP) or ORAC assay, would show whether the additive behaviour also holds for metal reduction and hydrogen-atom transfer, where synergy between herbal components is more often reported (Kurin et al., 2025; Yap et al., 2023). HPLC quantification of the major phenolic acids and flavones would identify the

constituents behind the close link between TPC and activity, and storage studies would establish how long the decoctions retain it.

Table 1. Observed values of the mixed decoctions compared with the values expected from an additive model of the two single-plant decoctions

RG:CG	Parameter	Observed	Expected	O/E	p	Interaction
75:25	TFC (mg QE/L)	23.12 ± 1.07	31.06 ± 1.29	0.74	0.001	Below additive
	TPC (mg GAE/L)	561.92 ± 8.90	539.32 ± 6.05	1.04	0.027	Above additive
	IC_{50} (µg/mL)	1266.90 ± 226.74	1210.25 ± 88.49	0.96	0.708	Additive
50:50	TFC (mg QE/L)	24.92 ± 1.13	33.22 ± 1.57	0.75	0.003	Below additive
	TPC (mg GAE/L)	502.69 ± 6.16	484.80 ± 6.33	1.04	0.025	Above additive
	IC_{50} (µg/mL)	1272.46 ± 45.70	1335.27 ± 98.67	1.05	0.379	Additive
25:75	TFC (mg QE/L)	49.90 ± 2.35	35.38 ± 2.12	1.41	0.001	Above additive
	TPC (mg GAE/L)	422.42 ± 26.02	430.27 ± 7.98	0.98	0.660	Additive
	IC_{50} (µg/mL)	1497.56 ± 64.29	1489.09 ± 140.35	0.99	0.931	Additive

Note. Data are mean ± SD (n = 3). Expected value $E = f{\cdot}X_{RG} + (1 - f){\cdot}X_{CG}$, where f is the mass fraction of red galangal. For IC_{50} the model was applied to activity ($1/IC_{50}$) and converted back, and O/E is the ratio of observed to expected activity, so that O/E > 1 indicates higher activity than expected. p, Welch's *t*-test of observed versus expected values. CG, citronella grass; O/E, observed-to-expected ratio; RG, red galangal; TFC, total flavonoid content; TPC, total phenolic content.

4. CONCLUSION

Decoctions of red galangal, citronella grass, and their mixtures all contained phenolic compounds and flavonoids. The plant ratio controlled each property measured: TPC and ABTS activity were highest in red galangal and declined as citronella grass increased, whereas TFC peaked at the 25:75 ratio. The radical-scavenging activity of every mixture equalled the value predicted from the two single-plant decoctions, so the two plants act additively, without synergy or antagonism, and the ratio adjusts antioxidant capacity in proportion to its red galangal content. Activity followed total phenolic content closely (r = 0.97) but not flavonoid content, which deviated from additivity without affecting activity. Because the activity of any blend can be predicted from its two components, the ratio offers a simple way to set the antioxidant capacity of a red galangal–citronella grass decoction, and these formulations can be developed further as functional herbal beverages.

AUTHOR CONTRIBUTIONS

Conceptualization, D.E.K., A.P.L. and A.F.; methodology, D.E.K. and A.F.; software, D.M.S.; validation, D.E.K.; formal analysis, D.M.S. and S.V.; investigation, D.E.K., D.M.S. and S.V.; resources, D.M.S. and S.V.; data curation, D.M.S., A.P.L. and A.F.; writing—original draft preparation, D.M.S. and S.V.; writing—review and editing, D.E.K.; visualization, D.M.S. and S.V.; supervision, D.E.K. and A.F.; project administration, D.E.K. and A.P.L.; funding acquisition, D.E.K. All authors have read and agreed to the published version of the manuscript.

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The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist in improving the clarity, structure, or readability of the text. The authors carefully reviewed and edited the manuscript and take full responsibility for the final content.

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