



The Synergistic Potential of Red Ginger (*Zingiber officinale* Roscoe var. *Rubrum*) and Acacia Honey in Herbal Formulation

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ABSTRACT

Red ginger (*Zingiber officinale* Roscoe var. *Rubrum*) and acacia honey are traditionally recognized for their health benefits, including their antioxidant properties, and are used traditionally to support lactation. Combining these two ingredients offers significant potential for developing herbal nutraceutical products. This study aimed to evaluate the antioxidant activity of a combination of red ginger and acacia honey at specific ratios. The formulation was created by mixing red ginger extract and acacia honey at two different ratios (50:50 and 70:30). The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method to determine the IC₅₀ value, while the flavonoid content was measured spectrophotometrically. The 50:50 formulation (Sample A) exhibited higher antioxidant activity, with an IC₅₀ value of 172.80 mg/L and a flavonoid content of 58.84 mg Eq Qe/100 g, compared to the 70:30 formulation (Sample B), with an IC₅₀ value of 268.91 mg/L and a flavonoid content of 40.80 mg Eq Qe/100 g. The combination of red ginger and acacia honey exhibits significant biological activity, with the ratio of ingredients affecting the antioxidant capacity and flavonoid content. Formulations with a 50:50 ratio show potential for development. However, clinical validation and product stability studies are recommended in future studies.

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Introduction

Breast milk is an ideal primary source of nutrients for babies because it contains all the necessary nutrients for optimal growth and development, including immunological and bioactive components that are not found in formulas.¹ However, challenges such as low breast milk production remain obstacles to achieving exclusive breastfeeding, particularly in developing countries like Indonesia.² Oxidative stress has been reported as one of the contributing physiological factors that may indirectly influence lactation performance.³ Therefore, effective, evidence-based interventions are needed to boost breast milk production and support the health of mothers and babies. In this context, the use of natural products with traditional applications in supporting lactation, particularly those derived from local plants, is of interest. Red ginger (*Zingiber officinale* Roscoe var. *Rubrum*) has been traditionally used in certain cultures as a galactagogue.⁴ Red ginger contains bioactive compounds such as gingerol and shogaol, which have antioxidant, anti-inflammatory, and antimicrobial properties.⁵⁻⁷ These activities may help reduce oxidative stress, potentially creating a physiological environment conducive to lactation; however, this requires further direct investigation. Red ginger is also known to affect hormone pathways, such as those involving prolactin, which plays a vital role in lactogenesis.⁸

Another promising local ingredient is acacia honey, which has a high flavonoid and phenolic content that supports antioxidant and antimicrobial activity.⁹⁻¹⁰ Previous animal and human studies have reported that consuming honey can increase levels of prolactin and estrogen.¹¹ The potential of red ginger and acacia honey as galactagogues has been reported; however, research into their combined use is limited. Furthermore, data on the effects of such formulations on physiological parameters, including prolactin levels, is scarce. Therefore, this study aimed to develop and characterize a red ginger–acacia honey formulation at two ratios (50:50 and 70:30, v/v) and to evaluate its antioxidant activity and total flavonoid content using spectrophotometric methods.

This approach is intended to contribute to the development of scientifically based herbal products, particularly those with potential relevance to maternal health. Additionally, the results of this study are expected to provide a scientific basis for considering the combination of red ginger and honey as a potential candidate for further investigation in relation to lactation support, while also broadening the use of local herbal ingredients in nutraceutical formulations.

Materials and Methods

Reagents and equipment

Anal grade ethanol (95% and 70%), acetate buffer solution (pH 4 and pH 7), methanol, ethyl acetate, DPPH (2,2-diphenyl-1-picrylhydrazyl), aluminium chloride (10%) and sodium acetate (1 M) were obtained from PT Merck Chemicals and Life Sciences, Indonesia and UV-Vis Spectrophotometer (DR3900) from Hach Malaysia SDN BHD, Indonesia.

Plant collection, identification, preparation and extraction

Fresh red ginger (*Zingiber officinale* Roscoe var. *rubrum*) and pure acacia honey (natural honey from *Acacia mangium* nectar produced by *Apis mellifera*) were collected in June 2024 from Malinau Seberang Village, Malinau Utara District, Malinau Regency, North Kalimantan, Indonesia (GPS coordinates: 3.5812° N, 116.6329° E). The plant material was taxonomically identified at Laboratorium Ekologi dan Konservasi Biodiversitas Hutan Tropis (Herbarium Mulawarman –

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HMMI) by a Taxonomist, Dr Ir. Rita Diana of the Faculty of Forestry and Tropical Environment, Universitas Mulawarman, Indonesia, where a voucher specimen with the number: 348/UN17.4.08/LL/2025 was deposited.

Plant preparation

The fresh red ginger (*Zingiber officinale* Roscoe var. *Rubrum*) plant was thoroughly washed with running water to remove dirt, then cut into small pieces (2–3 cm) and dried at room temperature for 72 hours, until the moisture content was less than 10%. The dried ginger samples were then ground into a fine powder with a particle size of ≤ 1 mm using a blender to give 100 g of the sample. A total of 50 g of the red ginger powder was extracted using the maceration method with 500 mL of 95% ethanol (1:10 w/v). The maceration process was carried out at room temperature (25 °C) for 48 hours, with continuous stirring using a magnetic stirrer to ensure homogeneity. Once complete, the solution was filtered using Whatman No. 42 filter paper to separate the solid residue. The filtrate was evaporated using a rotary evaporator at 40 °C to produce a thick extract. This extract was then stored at 4 °C until further analysis.

Acacia Honey Extraction

The pure acacia honey (natural honey from *Acacia mangium* nectar produced by *Apis mellifera*) was used for this experiment. The acacia honey (100 g) was mixed with 200 mL of distilled water and homogenized using a mechanical shaker at 200 rpm for 15 minutes. The resulting honey solution was extracted using ethyl acetate as solvent at a ratio of 1:3 (v/v). To enhance the efficiency of separating the active compounds, the extraction process was carried out using an orbital shaker for 30 minutes. The organic fraction (ethyl acetate) was then separated and filtered using Whatman No. 42 filter paper to remove any solids. The resulting filtrate was evaporated using a rotary evaporator at 40 °C until a concentrated honey extract was obtained. This extract was stored at 4 °C for formulation and subsequent analysis.

Product Formulation

The product formulation was prepared by mixing red ginger extract and acacia honey at two different ratios: 50:50 (v/v), referred to as Sample A, and 70:30 (v/v), referred to as Sample B. A homogenizer was used to ensure an even distribution of the ingredients during the mixing process. After homogenization, the formulation was diluted with distilled water to reach a total volume of 250 mL. Physicochemical stability was evaluated by measuring pH, viscosity, and total phenolic content, with pH measurements performed using a digital pH meter calibrated with pH 4 and pH 7 acetate buffers. The viscosity of the formulation was analyzed using a rotational viscometer at room temperature (25 °C).

Total phenolic content determination

The total phenolic content of the formulated samples was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm, employing the Folin-Ciocalteu method. In this assay, 1 mL of the formulated sample was mixed with 5 mL of Folin-Ciocalteu reagent and incubated at room temperature for 5 minutes. Then, 4 mL of a 7% sodium carbonate solution (w/v) was added, and the mixture was incubated in the dark at room temperature for 30 minutes to allow the complex formation reaction to occur. The absorbance of the solution was measured at a wavelength of 765 nm using a UV-Visible spectrophotometer. Total phenolic content was calculated using a gallic acid standard curve and expressed in mg GAE (gallic acid equivalents) per litre of sample. All measurements were performed in triplicate to ensure the validity and consistency of the data.

Antioxidant Activity Test

The antioxidant activity of the formulation was evaluated using the spectrophotometric DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which measures a sample's ability to reduce free radicals. A 0.1 mM DPPH solution was prepared by dissolving 3.94 mg of DPPH in 100 mL of 95% ethanol. 2 mL of the formulation solution was mixed with 2 mL of the 0.1 mM DPPH solution in a reaction tube. The mixture was then incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance of the solution was measured at a

wavelength of 517 nm using a UV-Vis spectrophotometer. The decrease in DPPH absorbance was calculated to indicate the inhibition of free radicals. The IC_{50} value, indicating the formulation concentration required to inhibit 50% of free radicals, was calculated using a linear regression equation between formulation concentration and inhibition percentage. The test was conducted in triplicate to ensure accuracy and consistency of results. As a lower IC_{50} value indicates higher antioxidant capacity, this parameter was used to compare the effectiveness of each formulation.

Total Flavonoid Test

The total flavonoid content was determined using the aluminium chloride ($AlCl_3$) reagent colorimetric method. The sample formulation solution (1 mL) was added to a 10 mL volumetric flask. The solution was then mixed with 3 mL of methanol p.a., 0.2 mL of a 10% aluminium chloride solution, and 0.2 mL of a 1 M sodium acetate solution. Distilled water was added until the mark on the flask was reached, and the mixture was thoroughly homogenized. The solution was then incubated in the dark at room temperature for 30 minutes to allow the flavonoid- $AlCl_3$ complex to form. After incubation, the absorbance of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 430 nm, the maximum wavelength for the flavonoid- $AlCl_3$ complex as determined by measuring the absorbance at wavelengths between 300 and 600 nm.¹² The total flavonoid content is expressed in milligrams of quercetin equivalent per 100 grams (mgEqQe/100 g), based on a quercetin standard curve created using a series of standard solutions with specific concentrations. All tests were performed in triplicate to ensure the results were valid and consistent.

Statistical Analysis

Data were presented as mean \pm SD of triplicated determination. A p-value of < 0.05 was considered significant.

Results and Discussion

Different compounds with biological activities have been identified in red ginger (*Zingiber officinale* Roscoe var. *Rubrum*) extracts, including gingerol, shogaol, 4-methoxybenzaldehyde, phenolic compounds, and flavonoids. These compounds are known to have antioxidant and anti-inflammatory properties. Additionally, the presence of aromatic compounds such as curcumen and (E, E)- α -farnesene was confirmed, further supporting the biological potential of red ginger as a functional ingredient. The dominant compounds in the water extract of red ginger are shogaol and citral, which support antioxidant activity and contribute to a distinctive aromatic character. The presence of these compounds in plants provides a robust scientific foundation for developing herbal formulations based on red ginger and acacia honey as functional products. The main bioactive compounds identified in red ginger and acacia honey extracts, along with their retention times and biological activities, are presented in Table 1.

The product formulation process involved mixing red ginger extract (*Zingiber officinale* Roscoe var. *Rubrum*) and acacia honey at two different ratios: 50:50 (Formulation A) and 70:30 (Formulation B). Red ginger extract was obtained by solvent extraction with a mixture of methanol and water to isolate primary bioactive compounds, including gingerol, shogaol, and other phenolic compounds. The acacia honey used in these formulations is pure honey that has been analyzed and found to contain phenolic compounds, flavonoids, and hydroxymethylfurfural (HMF). The chemical profile of acacia honey was further confirmed using GC-MS analysis, as shown in Figure 1. The two main ingredients are mixed using a high-speed homogenizer for five minutes at room temperature to ensure the solution is homogeneous. After mixing, the formulation is diluted with distilled water until it reaches a total volume of 250 mL. This formulation aims to produce a functional herbal beverage with good physicochemical stability and significant antioxidant activity, as evidenced by the physicochemical stability testing of the pH, viscosity, and total phenolic content of both formulations. The analysis revealed that both formulations had a pH within the optimal range for functional drinks (between 5.2 and 5.6). Formulation A has a pH of 5.6 ± 0.1 , while Formulation B has a pH of 5.2 ± 0.1 , indicating stable acidity during

formulation.

Evaluation of total phenolic content using the Folin-Ciocalteu method revealed that formulation A had a higher phenolic content of 210 ± 12 mg GAE/L compared to formulation B at 180 ± 15 mg GAE/L. The higher total phenolic content in formulation A indicates greater antioxidant potential than in formulation B, which may be due to a more balanced ratio of active ingredients in formulation A, thus allowing maximum contribution from the phenolic compounds in red ginger and *acacia* honey. Overall, the test results show that Formulation A was more stable and had greater antioxidant potential than Formulation B. The antioxidant screening results, which evaluated the free radical inhibition capabilities of red ginger and honey formulations using the DPPH (2,2-diphenyl-1-picrylhydrazyl), revealed that the antioxidant capabilities of each formulation varied, as represented by the IC_{50} value. The test results showed that the IC_{50} value of formulation A was 172.80 mg/L, which indicates that 0.17 g of formulation A in 1000 mL of solution can suppress 50 % of DPPH free radicals. At higher concentrations, formulation A achieves an inhibition efficiency of over 60 %. The antioxidant inhibition curve of formulation A is illustrated in Figure 3. These results suggest that formulation A exhibits significant antioxidant activity. Similarly, the IC_{50} value for formulation B was recorded as 268.91 mg/L, suggesting that 0.268 g of formulation B in 1000 mL of solution is required to reduce 50 % of DPPH free radicals. The antioxidant inhibition profile of Formulation B (70:30) is shown in Figure 4. Formulation B appears to have lower antioxidant activity than formulation A, with maximum free radical inhibition of only around 50 % at high concentrations.

Table 1: Mass Spectrometric (MS) Analysis Results of Red Ginger and *Acacia* Honey Extracts

Raw Material s	Main Compounds	Retention Time	Main Activities
Red Ginger	(+)-[6]-Gingerol	10.142	Antioxidant, anti-inflammatory
	Shogaol	10.137; 11.998	Antioxidant
	4-Methoxybenzaldehyde	12.879	Antioxidant
	Curcumin	12.634	Antioxidant, aromatic
	Cutral	3.713	Aromatic, Antioxidant
<i>Acacia</i> Honey	5-Hydroxymethylfurfural (HMF)	12.784	Quality and antioxidant indicators
	2-Furancarboxylic Acid	10.207	Antioxidant
	Cirsiumaldehyde	23.342	Antioxidant ²⁶
	Nonanoic Acid	17.122	Stability ²⁷

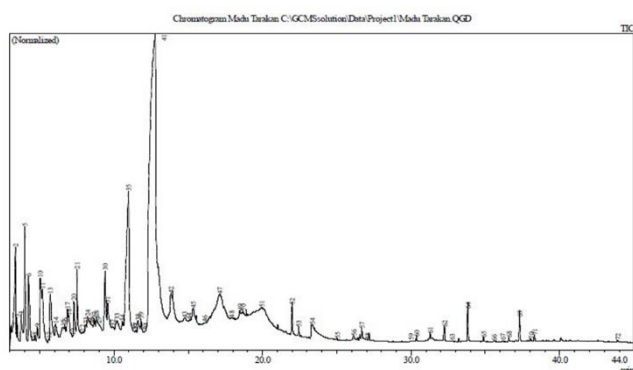


Figure 1: GC chromatogram of *Acacia* honey

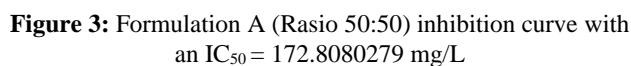
In comparison, formulation A, with a ratio of 50:50, has a lower IC_{50} value than formulation B, requiring a smaller amount to suppress 50 % of free radicals. This indicates that formulation A has higher antioxidant activity than formulation B, which may be due to the more balanced ratio of red ginger and *acacia* honey in formulation A. Red ginger is known to have an antioxidant mechanism through electron transfer. It plays a significant role in increasing antioxidant capacity, while the flavonoids and phenolic compounds in honey support this activity. These results suggest that formulation A has greater potential as an effective natural antioxidant source.

Total flavonoid testing aims to evaluate the content of flavonoid compounds in red ginger and *acacia* honey formulations, as this is an indicator of antioxidant capacity. The analysis was carried out using the aluminium chloride ($AlCl_3$) colorimetric method, which forms a stable complex with flavonoid compounds that is then measured at a wavelength of 430 nm using a UV-Vis spectrophotometer. The results showed a significant difference ($p < 0.05$) in total flavonoid content between Formulation A (50:50 ratio) and Formulation B (70:30 ratio). Formulation A had a flavonoid content of 58.84 ± 2.13 mg Eq Qe/100 g, whereas Formulation B had a lower flavonoid content of 40.80 ± 1.92 mg Eq Qe/100 g. The total flavonoid test results showed that formulation A (50:50) had a higher flavonoid content than Formulation B (70:30): 58.84 ± 2.13 mg Eq Qe/100 g and 40.80 ± 1.92 mg Eq Qe/100 g, respectively. This indicates greater potential for antioxidant activity in formulation A, which is consistent with the lower IC_{50} value observed in this formulation. The more balanced ratio of red ginger to *acacia* honey in formulation A is estimated to contribute significantly to the concentration of phenolic and flavonoid compounds. Bioactive compounds, such as gingerol and shogaol, in red ginger are known to possess antioxidant properties and play a crucial role in enhancing the formulation's potential biological activity. Due to its higher flavonoid content, Formulation A is the superior candidate for development as a functional beverage with enhanced antioxidant benefits and potential health-supporting properties. The total flavonoid content of both formulations is presented in Table 3.

This study highlights the importance of combining red ginger (*Zingiber officinale* Roscoe var. *Rubrum*) and *acacia* honey in an herbal Formulation (A, 50:50 ratio) that demonstrated a significantly lower IC_{50} value (172.80 ± 5.42 mg/L) than formulation B (70:30 ratio) (268.91 ± 6.37 mg/L), indicating a greater antioxidant capacity. This increased activity is likely due to the synergistic effects of gingerol and shogaol from red ginger, which can neutralize free radicals through electron donation and hydrogen transfer. Additionally, flavonoids and phenolic compounds in *acacia* honey support antioxidant activity by binding to free radicals and enhancing antioxidant enzyme pathways. Such synergy suggests that the balance between red ginger and honey markedly influences the formulation's biological activity. The red ginger compounds gingerol and shogaol are known to neutralize free radicals through electron donation and hydrogen transfer mechanisms, and their presence in the red ginger extract was confirmed by GC analysis, as shown in Figure 2. This protects against oxidative damage to cell membranes, proteins, and DNA caused by reactive oxygen species.¹³ Gingerol can reduce the rate of lipid peroxidation, a key indicator of oxidative stress in biological systems. Additionally, *acacia* honey contains flavonoids and phenolic compounds that support antioxidant activity by binding to free radicals and regulating endogenous antioxidant enzyme pathways. The flavonoids quercetin and kaempferol, found in honey, are known to enhance the activity of the enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx), which play a crucial role in maintaining the body's redox balance.¹⁴ The combination of formulation A produces a synergistic effect, enhancing antioxidant capacity. Previous studies have shown that mixtures of phenolic compounds from various sources can be more biologically effective than single ingredients.¹⁵



The physicochemical stability parameters of both formulations, including pH and total phenolic content, are summarized in Table 2. The physicochemical stability of the formulations supports the development of herbal-based functional beverages. The pH values of Formulations A and B were 5.6 ± 0.1 and 5.2 ± 0.1 , respectively, both of which are within the optimal range for herbal products. This moderate acidity protects against contamination by pathogenic microorganisms, ensuring product safety during storage.¹⁶ Formulation A had a higher total phenolic content (210 ± 12 mg GAE/L) than Formulation B (180 ± 15 mg GAE/L). This higher content directly contributed to greater antioxidant capacity, as evidenced by the lower IC₅₀ value. The dominant phenolic compounds in red ginger, gingerol and shogaol, play a crucial role in enhancing redox stability and reducing inflammation by inhibiting the NF- κ B pathway.¹⁷ Conversely, the high honey content in formulation B has the potential to increase the water content and reduce the concentration of active phenolic compounds per unit volume.¹⁴ It has the potential to support lactation by reducing oxidative stress and inflammation. The gingerol found in red ginger has significant anti-inflammatory properties. It works by inhibiting the expression of pro-inflammatory cytokines, such as TNF- α and IL-6, while increasing the expression of adiponectin. Adiponectin is vital for metabolic regulation during lactation.¹⁸ This effect optimizes mammary gland function and supports physiological milk production. Previous studies have shown that the combination of red ginger and honey can boost prolactin and estrogen levels by reducing oxidative stress.¹⁹ These two hormones play an essential role in stimulating milk production and improving the structure of mammary tissue. Due to its higher antioxidant capacity and phenolic content, Formulation A shows greater potential than Formulation B for supporting the holistic health of breastfeeding mothers.



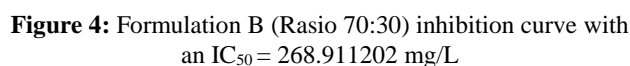
viscosity, indicative of a higher concentration of phenolic and Flavonoid compounds.

Table 2: Physicochemical Stability of the formulated products.

Parameter	Formulation A (50:50)	Formulation B (70: 30)
pH	5.6 ± 0.1	5.2 ± 0.1
Total Phenolic (mg GAE/L)	210 ± 12	180 ± 15

NOTE: This table compares the physicochemical parameters of the two formulations, including pH and total phenolic content.

Using local ingredients such as red ginger and *acacia* honey provides health benefits and supports local food security and community-based health promotion. Beal *et al.* (2018) found that products based on local natural ingredients are more affordable and environmentally friendly and can empower local communities.¹⁶ This study provides valuable insights into the effectiveness of combining local herbs, such as red ginger and *acacia* honey, to support lactation and boost antioxidant capacity. The results show that formulation A has an IC₅₀ lower than that of formulation B. This indicates the high scavenging capacity of the formulation due to the low amount of radical scavengers required from the formulation to reduce DPPH.²⁰ The primary bioactive compounds in red ginger, gingerol and shogaol, are known to neutralize free radicals through electron and hydrogen transfer mechanisms, thereby protecting biomolecules from oxidative damage.¹⁷ On the other hand, *acacia* honey supports antioxidant activity through its Flavonoid and phenolic content. These compounds bind free radicals and enhance the regulation of endogenous antioxidant pathways, increasing the activity of enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx).¹⁴ The combination of the two ingredients in formulation A demonstrates a synergistic effect, supporting the hypothesis that a mixture of phenolic-based active ingredients produces greater antioxidant activity than a single ingredient.¹⁹ This study sheds light on the effectiveness of combining local herbs, such as red ginger and honey, to support lactation and boost antioxidant capacity. The higher antioxidant activity of formula A indicates that the balance between components in a formulation plays a key role in determining its biological properties. Therefore, it is expected to be a source of antioxidants that support prolactin production. The flavonoids and phenolic compounds in honey reinforce this role by supporting antioxidant activity through free radical binding mechanisms.^{9,21} Red ginger is effective due to its anti-inflammatory properties, which are derived from gingerol. These properties help to reduce inflammation and oxidative stress, thereby supporting optimal physiological conditions for breast milk production.²² Meanwhile, previous research by Usman *et al.* (2021) also shows that the combination of ginger and honey can affect prolactin and estrogen levels by reducing oxidative stress, which supports the theory that these ingredients work together to promote lactation.¹⁹



This figure presents the inhibitory curve of the herbal formulation with a 70:30 (v/v) ratio of red ginger to *acacia* honey. The formulation appears lighter in color and slightly less viscous compared to Formulation A.

Table 3: Total Flavonoid Content in the formulated products.

Formulation	Total Flavonoid (mgEqQe/100 g) \pm SD
Formulation A (50:50)	58.84 \pm 2.13
Formulation B (70:30)	40.80 \pm 1.92

NOTE: This table presents the total flavonoid content of the two formulations, expressed in milligrams of quercetin equivalents per 100 grams (mg Eq Qe/100 g)

The fluctuation in antioxidant capacity and hormonal effectiveness in this formulation demonstrates that different proportions of ingredients can produce distinct biological effects. Although the phenolic compounds dominant in honey support the hormonal pathway, they contribute only minimally to antioxidant activity in the 70:30 formulation. This can be explained by honey's high-water content, which reduces the phenolic concentration per unit volume.^{9,24} Further research is needed to evaluate the effect of variations in ingredient ratios on the stability of the active compound, its bioavailability, and the duration of its effect in this formulation. From a practical standpoint, these findings present opportunities to develop two types of herbal nutraceutical products. Both have great potential for use in breastfeeding health programmes, particularly in regions where access to synthetic supplements is limited. Additionally, using local herbal ingredients such as red ginger and *acacia* honey can strengthen regional food security and promote community-based health.²⁵

Conclusion

This study demonstrates the significant potential of the combination of red ginger (*Zingiber officinale* Roscoe var. *Rubrum*) and acacia honey in potentially supporting lactation and increasing antioxidants. Red ginger acts as an antioxidant and anti-inflammatory agent due to its gingerol and shogaol content. This is achieved through electron transfer mechanisms and the inhibition of inflammatory pathways. Meanwhile, acacia honey supports antioxidant activity by binding free radicals and regulating endogenous antioxidant enzymes through its Flavonoid and phenolic content. Together, these two ingredients create a formulation with superior biological properties that support the hormonal mechanisms of prolactin and estrogen during lactation.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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