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Antioxidant, phytochemical, and proximate test in cookies fortified with *Polyscias scutellaria* leaves on development snack for breastfeeding mother

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ABSTRACT

Hypogalactia is a condition characterized by low milk production. *Polyscias scutellaria* has been traditionally used to increase breast milk production among breastfeeding mothers in East Java, Indonesia. This study aimed to evaluate the antioxidant activity, phytochemical content, and proximate composition of cookies fortified with *P. scutellaria* leaves. The cookies were divided into three types: regular cookies (RC), which contain gluten and egg; gluten-free cookies (GFC); and gluten-free, egg-free cookies (GFEFC). The antioxidant activity was measured using the 1-diphenyl-2-picrylhydrazyl (DPPH) assay, while phytochemical contents were evaluated by total phenolic content (TPC) and total flavonoid content (TFC). The proximate analysis included total ash, moisture, fat, protein, carbohydrate, and starch content. The antioxidant activity results showed that RC, GFC, and GFEFC reached inhibition activity for DPPH assay of 52.17%, 67.93%, and 64.70%, respectively. GFC had the highest TPC, while GFEFC had the highest TFC. The RC cookies had the highest carbohydrate and protein content, while the highest fat content was found in GFEFC. Proper nutrition is essential for breastfeeding mothers and infants to ensure adequate nutrition for the infant and energy for the mother. In conclusion, cookies fortified with *P. scutellaria* can be used as a snack for breastfeeding mothers.

Contribution/Originality: This study originally used *Polyscias scutellaria* leaves to develop lactation cookies for breastfeeding mothers. This is the first research on exploring the benefits of *P. scutellaria* to enhance the nutritional value of cookies for lactation. The nutritional information of lactation cookies includes proximate analysis, antioxidant activity, and phytochemical content.

1. INTRODUCTION

Breast milk is considered the gold standard of newborn feeding due to its exceptional capacity to supply all vital nutrients and antibodies, as well as its various health advantages (Zhang et al., 2021). Breast milk is composed of an optimal combination of nutrients, such as proteins, lipids, carbohydrates, vitamins, and minerals, that are specifically developed to meet the nutritional needs of growing infants (Martin, Ling, & Blackburn, 2016). Breast milk is abundant in immunological components, enzymes, and antibodies that protect newborns from infections and diseases (LeMaster et al., 2023), minimizing susceptibility to gastrointestinal infections, lung infections, ear infections, and allergies (Palmeira & Carneiro-Sampaio, 2016).

Exclusive breastfeeding (EBF) for 6 months, followed by continued complementary feeding until 2 years of age, is strongly recommended by the World Health Organization (WHO) to provide complete nutrition for infants (Laksono, Wulandari, Ibad, & Kusrini, 2021). EBF increases infant morbidity, especially to prevent diarrhea and acute respiratory infections, which are the most common infectious diseases (Hossain & Mihrshahi, 2022). Production of breast milk is affected by several hormones, with prolactin and oxytocin having direct effects on milk secretion. Prolactin stimulates milk synthesis in the mammary glands, while oxytocin triggers the let-down reflex, allowing the milk to be ejected (Sumirah Budi Pertami et al., 2024). However, breast milk production also involves some hormones via indirect mechanisms, especially estrogen and progesterone. These reproductive hormones play key roles in mammary gland development, particularly during puberty, pregnancy, and lactation. Estrogen induces prolactin secretion from the anterior pituitary and prolactin receptors in the epithelium of the mammary gland. The effects of estrogen have been associated with low milk supply in breastfeeding women (Jin, Perrella, Lai, Taylor, & Geddes, 2024). Estrogen promotes further ductal elongation and branching, while progesterone drives the development of alveolar buds.

Some breastfeeding women experience a condition called hypogalactia, which refers to reduced milk production (Pertami, Arifah, Atho'illah, & Budiono, 2021). This condition can result in an inadequate milk supply, making it difficult to fulfill an infant's nutritional requirements and potentially affecting their growth and development (Budiono, Pertami, Arifah, & Atho'illah, 2023). Various factors may contribute to this issue, such as hormonal disruptions, underdeveloped breast tissue (McBride et al., 2021), specific health conditions, improper breastfeeding practices, maternal stress or exhaustion, and poor dietary intake or dehydration in the mother (Bao et al., 2023; Laura et al., 2019). Treatment options for hypogalactia in breastfeeding women vary based on the underlying causes and typically involve a comprehensive approach, which may include both pharmaceutical galactagogues and herbal remedies. Herbal galactagogues are natural substances believed to stimulate milk production in lactating mothers. Due to their cultural familiarity and perceived safety, many mothers choose to use these herbal supplements as part of their lactation strategy (McBride et al., 2021). Several herbal galactagogues are commonly used to enhance breast milk production, including fenugreek (*Trigonella foenum-graecum*), katuk (*Sauropus androgynus*), milk thistle (*Silybum marianum*), and *Moringa oleifera* (Budiono et al., 2023). Additionally, extracts from *P. scutellaria* leaves have shown promise in increasing levels of prolactin and oxytocin in lactating rats (Budiono et al., 2023). Previous in silico studies have also suggested that the bioactive compounds in this plant may act as dopamine D2 receptor inhibitors (Budiono, Pertami, Arifah, & Lestari, 2021), and they may stimulate the serotonin 5-HT2A receptor (Pertami et al., 2021). Lactation cookies (LC) are food products that contain herbal galactagogues and have been widely used to help increase breast milk production. These cookies improve the flavor profile of galactagogues, potentially increasing maternal compliance. However, research on LC remains scarce. This study focused on formulating and evaluating a lactation cookie enriched with *P. scutellaria* leaves, examining its antioxidant properties, phytochemical content, and nutritional composition.

2. MATERIALS AND METHODS

2.1. Material

P. scutellaria leaf was purchased in a local market in Malang City, East Java, Indonesia. *P. scutellaria* leaf was taxonomically identified by a botanist expert with voucher specimen number 074/273A/102.7/2020 at UPT Laboratorium Herbal Materia Medica, Batu, East Java, Indonesia.

2.2. Preparation of Cookies

The cookies were divided into three types: regular cookies, gluten-free cookies, and gluten-free egg-free cookies. Each type of cookie contained 1% of *P.scutellaria* leaves powder. The ingredients for each cookie are presented in Table 1.

Table 1. The ingredients of each type of cookie.

Type of cookies	Type of flour	Weight of flour (Gram)	Sugar (Gram)	Eggs	Emulsifier (Spoon)	Maizena (Gram)	Butter (Gram)
RC	Wheat flour	200	150	2	1/4	50	200
GFC	Rice flour	200	150	2	1/4	50	200
GFEFC	Rice flour	200	150	0	1/4	50	250

Note: RC, Regular cookies; GFC, Gluten-free cookies; GFEFC, Gluten-free egg-free cookies.

2.3. Cookies Extraction

The lactation cookies were extracted using the following procedure as described by Najjar et al. (2022) with slight modifications. One gram of LC was mixed with 5 mL of distilled water and shaken for 5 minutes. The mixture was incubated for 30 minutes at 4°C, then centrifuged at 4500 rpm for 20 minutes Najjar et al. (2022). The supernatant was collected and stored at -20°C for further analysis.

2.4. Antioxidant Activity

The antioxidant activity was measured using the 1-diphenyl-2-picrylhydrazyl (DPPH) assay with slight modifications. 200 µL of cookie extract were mixed with 1 mL of DPPH solution (50 µM), then incubated for one hour at room temperature in the dark. The samples were measured at a wavelength of 517 nm. DPPH solution was used as a control. The percentage of inhibition was calculated using the following equation (Makhlouf et al., 2024).

$$\% \text{ Antioxidant activity} = \frac{A_{control} - A_{sample}}{A_{control}} \text{ x } 100$$

2.5. Total Phenolic Content (TPC) Assay

The Folin-Ciocalteu method, as described by Budiono et al. (2023), was used to assess total phenolic content (TPC). Briefly, 80 µL of sample or standard were added with 400 µL of 10x diluted Folin-Ciocalteu reagent, incubated for 5 minutes at room temperature. The mixture was then added with 300 µL of sodium carbonate (Na_2CO_3) at a concentration of 75 g/L, incubated for 2 hours in the dark at room temperature. The standard reagent used gallic acid to obtain a linear standard curve with concentrations ranging from 200 to 1000 µg/mL. Absorbance was measured at 740 nm. The results were expressed as gallic acid equivalents (GAE) in mg GAE per gram of dry extract.

2.6. Total Flavonoid Content (TFC) Assay

Total flavonoid content (TFC) was determined using an aluminum chloride colorimetric assay (Budiono et al., 2023). 250 µL of samples or standard was combined with 350 µL of distilled water and 75 µL of 5% sodium nitrite ($NaNO_3$) solution. The mixture was then added with 75 µL of 10% aluminium chloride ($AlCl_3$) solution. The mixture was left to incubate for 6 minutes at room temperature. Next, 500 µL of a 1 M sodium hydroxide (NaOH) solution was added. The final mixture was then evaluated using a UV-Vis spectrophotometer at a wavelength of 510 nm. Quercetin is prepared in concentrations ranging from 200 to 1000 µg/mL and served as the standard for establishing a calibration curve. The results were reported as quercetin equivalents (QE), mg QE/g of dry extract.

2.7. Proximate Test

The proximate analysis included the assessment of total ash, moisture, fat, protein, and carbohydrate contents. The determination of ash content was conducted using the dry ashing technique (Liu, 2019), while moisture content was measured through thermogravimetric testing (Park et al., 2022). The total fat content was quantified using the Soxhlet method (Hewavitharana, Perera, Navaratne, & Wickramasinghe, 2020). Total protein levels were determined using the Kjeldahl method (Goulding, Fox, & O'Mahony, 2020). The carbohydrate levels were calculated based on the equation.

% carbohydrate = 100% - % (ash + moisture + fat + protein) (Emelike, Ujong, & SC, 2020)

2.8. Data Analysis

Descriptive statistics were used to evaluate the proximate composition data. Antioxidant activity, total phenolic content (TPC), and total flavonoid content (TFC) were analyzed using one-way analysis of variance (ANOVA), with Duncan's Multiple Range Test (DMRT) employed for post hoc comparisons. A significance level of $p < 0.05$ was used to determine statistically significant differences among groups. All results were expressed as the mean ± standard deviation.

3. RESULTS

3.1. Evaluation of Phytochemical and Antioxidant Activity in Cookies Enriched with P. Scutellaria Leaves

The antioxidant activities of RC, GFC, and GFEFC were 52.17%, 67.93%, and 64.70%, respectively (Table 2). GFC has the highest antioxidant activity (67.93%) and the highest phenolic content (201.31 mg GAE/g). RC also reported the lowest antioxidant activity, TPC, and TFC. In the TFC assay, GFEFC has a higher content compared with GFC. However, all types of cookies achieved antioxidant activity >50%. These results suggest that GFC could be a candidate for lactation cookies based on antioxidant and phytochemical content.

Table 2. Antioxidant and phytochemical content.

Type of cookies	Antioxidant activity (%)	TPC (mg GAE/g)	TFC (mg QE/g)
RC	52.17±3.26	129.64±2.27	651.74±6.59
GFC	67.93±4.08**	201.31±2.28*	841.95±49.34*
GFEFC	64.70±1.37*	111.52±3.56	904.78±58.26**

Note: RC, Regular cookies; GFC, Gluten-free cookies; GFEFC, Gluten-free egg-free cookies.
*indicated a significant difference, **indicated significantly difference compared with *

3.2. Rich-Nutrition Value in Cookies Enriched with P. Scutellaria Leaves

Figure 1 illustrates the proximate test analysis. The RC type, which contains gluten and egg, has 5.33% protein and 71.1% carbohydrate, whereas GFC has 2.26% protein and 69.31% carbohydrate. Furthermore, GFEFC has 2.49% crude protein and 53.65% carbohydrate. The results from the proximate test indicate that RC has the highest carbohydrate and protein content. These results suggest that RC contains more energy than GFC and GFEFC. During lactation, a breastfeeding mother requires more nutrition and energy to produce adequate milk for infants.

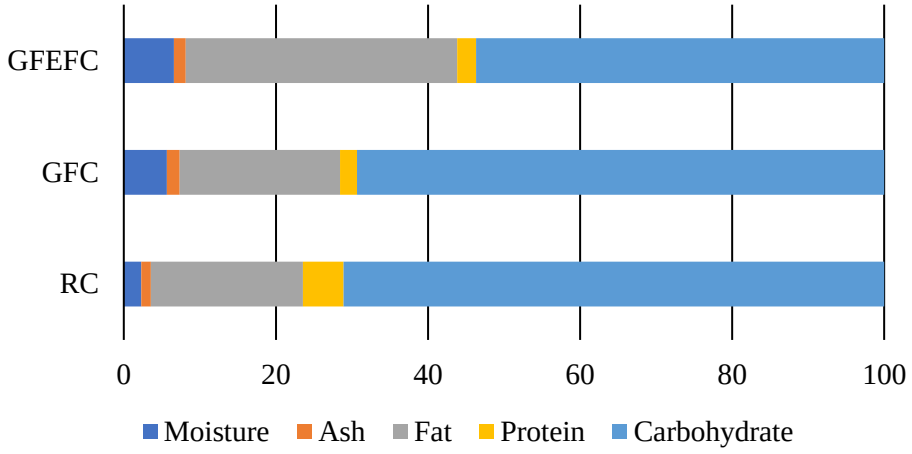

Figure 1. Proximate analysis result.

4. DISCUSSION

Breastfeeding is a vital aspect of infant development and maternal health, providing numerous benefits to both mother and infant. Various factors influence the composition of breast milk, a complex and dynamic fluid (Ford, Underwood, & German, 2020). The macronutrients such as fat, protein, and lactose undergo significant changes during lactation (Martin et al., 2016). One challenge among some breastfeeding mothers is low milk supply, which can be addressed through the use of herbal galactagogues. Lactation cookies have recently become popular snacks for nursing mothers due to their potential ability to improve milk production and provide nutrition for both mother and baby (Chumroenvidhayakul, Thilavech, Abeywardena, & Adisakwattana, 2023). In most cases, these cookies are made with various ingredients, while others add herbal galactagogues to maximize their health-promoting properties. A previous study using in silico and in vivo methods revealed that *P. scutellaria* leaves can be used as herbal galactagogues, meaning that the consumption of shield aralia helps increase breast milk production (Budiono et al., 2021; Sumirah B Pertami et al., 2021).

Based on the antioxidant activity, all types of cookies fortified with *P. scutellaria* leaves reached an antioxidant activity of more than 50%. The antioxidants exhibited potential free radical scavenging ability and reducing power, probably because they contained various phytochemicals (Sánchez et al., 2021). On measuring TPC and TFC, it was apparent that the cookies were rich sources of these bioactive compounds (Ford et al., 2020). Lactation cookies with antioxidants are beneficial for breastfeeding mothers, as they help reduce oxidative stress, boost the immune system, and improve overall wellness (Karbasi et al., 2022). Maternal diet rich in antioxidants also enhances breastmilk quality, sustains high levels of stamina, and promotes overall health. Furthermore, antioxidant-rich foods play an essential role in the optimal growth and development of infants. Therefore, it can be inferred from this study that cookies fortified with *P.scutellaria* may possess galactagogue properties, as they exhibit strong antioxidant activity and contain various bioactive compounds such as phenolics and flavonoids (Peter-Ikechukwu, Kabuo, Uzoukwu, Chukwu, & Ogazi, 2020). These constituents are important in modulating the immune response or enhancing lactogenic effects (Sánchez et al., 2021).

The proximate composition analysis of shield aralia lactation cookies provides insights into the simulated breast milk nutritional profile, thereby assisting mothers in maintaining an adequate milk supply. The analysis revealed that the lactation cookies are a rich source of macronutrients, including carbohydrates, proteins, and fats. Carbohydrates supply the energy needed for milk production and overall health in lactating mothers (Sánchez et al., 2021). Consuming carbohydrates helps maintain stable blood sugar levels, preventing fatigue and hypoglycemia, which is particularly important for breastfeeding mothers with increased energy demands (de Amorim, Rodrigues, Sussi, & Neri, 2024). Carbohydrate-rich foods also contain essential vitamins, minerals, and fiber. Adequate fiber intake from carbohydrate-rich foods supports digestive health and regular bowel movements, which can be particularly beneficial postpartum. Glucose in carbohydrates is diverted into lactocytes to maintain milk production (Stuebe, 2015).

On the other hand, breastfeeding mothers require protein for health and also provide high-quality milk to support infant growth. Breast milk contains important proteins such as casein and whey. These proteins are crucial for an infant's development and growth due to the role of amino acids in muscle tissue formation, repairing damaged tissues, and enabling general body growth of the infant (Wati, Sargowo, Nurseta, & Zuhriyah, 2023). Inadequate protein intake could inhibit infant growth, which is strongly associated with stunting in children (Semba et al., 2016). Proteins help produce digestive enzymes and hormones associated with lactation, such as prolactin and oxytocin. Therefore, consuming sufficient quantities of protein is important to support milk synthesis (Rasmussen et al., 2020). Breast milk also contains antibodies known as immunoglobulins, which prevent infants from getting infections.

5. CONCLUSIONS

This study showed that three types of cookies fortified with *P.scutellaria* have antioxidant activity >50%. The TPC and TFC assays indicated that the cookies contain bioactive compounds from phenolic and flavonoid groups.

The proximate test also suggests that *P.scutellaria* cookies provide macronutrients for breastfeeding mothers. These results from antioxidant activity, phytochemical content, and proximate analysis indicate that cookies fortified with *P.scutellaria* leaves can be used as lactation cookies for breastfeeding mothers to increase the quality and quantity of breast milk production.

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