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Total Flavonoid and Antioxidant Activity of Food Bar Torbangun – Katuk on The Effectiveness of Breast Milk Production

Total Flavonoid dan Aktivitas Antioksidan Food Bar Torbangun – Katuk terhadap Efektivitas Produksi ASI

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Antioxidant activity, Breast milk, Katuk, Torbangun, Total flavonoids

ABSTRACT

Background: The main reason for discontinuing exclusive breastfeeding is the low milk supply. Torbangun and Katuk are galactagogues that can stimulate milk production and be modified into food bars to increase breast milk production. Modifying the food bar using Torbangun and Katuk is food fortification to increase milk production for breastfeeding mothers.
Objectives: This study aimed to identify the total flavonoids and antioxidant activity of Torbangun flour, Katuk flour, and food bar Torbangun Katuk products and to analyze the effectiveness of Torbangun Katuk food bar in breastfeeding mothers
Methods: This was pre-experimental with a pre-test and post-test design to analyze milk production before and after consuming food bars. Raw materials and food bar products were analyzed for total flavonoids with aluminum chloride ($AlCl3$) and antioxidant activity using the DPPH (1,1-diphenyl-2-picrylhydrazil) method. The food bar had 2.5% Torbangun flour and 5% Katuk flour. The effectiveness test was conducted on ten breastfeeding mothers. Effectiveness was seen from changes in breast milk volume before and after consuming food bars with pairing T test for analysis.
Results: chemical analysis showed that the total flavonoids of Torbangun and Katuk flour were 4.06 mg/g and 5.30 mg/g, and antioxidants in Torbangun and Katuk flour were 39.77 ppm and 307.96 ppm, respectively. Furthermore, the total flavonoids and antioxidant activity of the Torbangun Katuk food bar product were 0.36±0.06 mg/g and 116.01±36.83 ppm, respectively. The effectiveness results showed an increase of 54.98% between the average breast milk volume before and after consuming food bars. Giving food bars significantly affected breastmilk production ($p<0.05$).
Conclusions: Torbangun flour and Katuk flour have the potential as food additives to increase breast milk production.

INTRODUCTION

Breast milk (ASI) is the best natural food for infants because it contains the energy and substances needed in the first six months of life[1]. Breastfeeding is essential for infants because it is the only intake supporting their nutrition in the first 1000 days of life (1000 HPK). Nutritional status at 1000 HPK can significantly impact health, intelligence, and productivity as the baby grows[2].

Although it is significant for the growth of infants, the rate of exclusive breastfeeding in Indonesia is still lacking[3]. The 2019 Indonesia Health Profile noted that the coverage of exclusive breastfeeding nationally was 67.74%[4]. In the 2018 Indonesian Basic Health Research, 65.7% of infants aged 0-23 months had never been breastfed because the mother's milk did not come out[5]. One of the common reasons for discontinuing exclusive breastfeeding is that mothers experience breastfeeding problems such as insufficient milk production[6]. Lack of milk production can occur due to nutritional and non-nutritional (maternal health, endocrinology, and mother's lifestyle)[7]. These factors can modulate physiological actions that regulate situations such as hypogalactias, which can be a problem with reduced milk production in the mother[8]. According to Anipindi et al. (2014), the factors that significantly influence breast milk production are hormones, especially prolactin[9]. Decreased prolactin response can inhibit milk secretion.

Stimulation of increased milk production can be done by increasing the intake of nutritional sources of galactagogue. A galactagogue is a substance of pharmaceutical agent, food, or herbal supplement) that can stimulate, maintain and increase the synthesis of milk[7,10]. Generally, galactagogues are divided into pharmacological galactagogues (conventional drugs) and natural galactagogues (nutrients derived from herbs)[11]. Conventional drugs used clinically to increase milk

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production are domperidone, metoclopramide, sulpiride, chlorpromazine, oxytocin, recombinant bovine somatotropin (rBST), medroxyprogesterone and thyrotropin-releasing hormone[6,7]. They block dopamine receptors in the central nervous system to increase prolactin synthesis by lactotroph cells in the anterior pituitary[7,12]. Pharmacological galactagogues are efficacious in breast milk production because they can increase serum prolactin levels, facilitating milk supply and production.

Nevertheless, conventional medicine has side effects on the mother. High doses of domperidone can cause gastrointestinal, headache, and increased risk of heart problems[12]. In addition, metoclopramide can cause side effects on the central nervous system, such as involuntary movements, dizziness, and headaches[13].

Galactagogue is believed that its use is more effective and safer, although some of the data obtained has not been clinically tested[14]. Nursing mothers prefer natural galactagogues because they are considered safer and more accessible to obtain[10,12]. Natural galactagogues vary from country to country depending on culture, beliefs, and biodiversity. Breastfeeding mothers in Hong Kong consume papaya soup (Carica papaya L.) to increase milk production. For the same reason, nursing mothers in the Philippines eat moringa soup (Moringa oleifera), nursing mothers in India consume turmeric (Curcuma longa) and Shatavari (Asparagus racemosus) soup, and breastfeeding mothers in Thailand eat basil (Ocimum basilicum)[15]. Several plants, such as fenugreek (Trigonella foenum-graecum), fennel (Foeniculum vulgare), and blessed thistle (Cnicus benedictus) are also reported to provide benefits in increasing milk production[16]. Indonesia has various natural potentials as a source of natural galactagogues, such as Torbangun leaves (Coleus ammbonicus Lour)[17,18] and Katuk leaves (Sauropus androgynus)[19,20].

Turkilmaz et al.'s research. (2011) stated that the effects of natural galactagogues are thought to be mediated by phytoestrogens and several other molecules that have effects similar to 17β-estradiol (E2), which is endogenous estrogen that can increase the proliferation of MEC (mammary epithelial cells)[21]. Some phytoestrogens are diosgenin, anethole estragole, shatavarine silybin, kaempferol, quercetin, silydianin, silychristin[7]. Damanik et al. (2017) found that in Torbangun leaves, there are digiprolactone and kaempferol derivatives (flavonoids of the flavonol group)[22,23], while Katuk leaves contain isoflavones and quercetin as phytoestrogens[24]. The galactagogue mechanism of phytoestrogens occurs in anterior pituitary lactotrophic cells and the mammary glands. In lactotrophic cells, phytoestrogens can increase gene expression and prolactin secretion (PRL) directly by E2R and indirectly by mE2R by increasing D2R inhibition. Furthermore, in the mammary glands, there is an increase in milk synthesis, continuity of gene expression, and cell proliferation[7]. From the research conducted by Damanik et al. (2017) found that ethyl acetate in Torbangun, kaempferol, and phytoestrogen molecules did not affect PRL. However, it is thought to affect the increase in prolactin receptors (PRLR), casein production, and lactose synthesis activity in the MEC so that it can increase milk production because the interaction of PRL and PRLR in epithelial glandular membrane cells can produce large milk production[23].

In addition, Torbangun and Katuk leaves have high antioxidant activity[25–27]. In the DPPH test (1,1-Diphenyl-2-Picrylhydrazil), Katuk leaves have potent antioxidant activity, as seen from the IC50 value of 32.04 ppm28 Torbangun leaves for 48.23 ppm[26]. The content of antioxidants in food can play a role in increasing milk production[29]. Antioxidants can improve the function of alveolar cells in the udder so that the epithelium is well maintained[30]. The study conducted by Manna et al. (2013) also confirmed the existence of a potential effect of antioxidant activity which can stimulate milk secretion through the regulation of the hormone prolactin, although the exact cause is unknown[31].

The combination of Torbangun and Katuk leaves increases milk production higher than when consumed separately[32]. Zakaria's study (2012) on Etawa breed goats stated that a combination of 2.5% Torbangun leaves with 2.5% Katuk leaves could increase milk production higher than the group given Torbangun leaves (5%) or Katuk leaves (5%)[32]. The substance can increase milk production in goats like humans, galactagogue[7,21,30]. Although research on the combination of Torbangun leaves and Katuk leaves were still on animals, it has been able to provide the potential to increase and facilitate breast milk.

Torbangun and Katuk leaves have a bitter taste when consumed directly[33,34]. Torbangun and Katuk leaves contain saponin compounds[23,34]. It is stated that the compound has a bitter taste[33]. Food acceptability can be increased by processing I to become a food bar. *Foodbar* is solid food formulated with high calories and made with various food ingredients (blended food), then formed into a solid and compact form (a food bar form) with the help of binders such as syrup, nougat, caramel, and chocolate[35]. The food bar was chosen because it is easy to make, can be combined with various food ingredients, has a long shelf life, has a sturdy structure, and is not easily broken and not easily brittle[36]. The acceptability can be improved by processing Torbangun and Katuk into food bars because it allows for adding other ingredients in the manufacturing process and makes it easier for breastfeeding mothers to consume them.

Therefore, this study aimed to process Torbangun and Katuk leaves into flour as the main ingredient for Torbangun and Katuk food bars. Apart from that, Torbangun and Katuk flour were analyzed for total flavonoids and their antioxidant activity to support their potential as breast milk enhancers. Furthermore, an analysis of the total flavonoid content and antioxidant activity in processed food bar products was carried out and tested the effectiveness of food bars for breastfeeding mothers.

METHODS

This study used a pre-experimental research design to see the effectiveness of breast milk production after consuming Torbangun Katuk food bar products. The design used was the pre and post-test design, namely to measure the level of change in the volume of breast milk

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of breastfeeding mothers who were given the Torbangun Katuk food bar as a result of the treatment.

The study consisted of the process of making Torbangun and Katuk flour, analysis of total flavonoids and antioxidant activity of Torbangun Katuk flour, making Torbangun Katuk food bars, analysis of total flavonoids and antioxidant activity of Torbangun Katuk food bars and testing the effectiveness of Torbangun Katuk food bars on ten breastfeed mothers. This study was approved (ethical approval) by KEPK of the Jakarta Veterans National Development University (No:2836/XII/2020/KEPK).

Flour making of Torbangun Katuk leaves held at the SEAFAST Center IPB University. The flooring method used for the two leaves is drying using a drum dyer. The flouring stage began by separating the leaves from the stems and sorting them; only fresh and young leaves were used. Then the leaves were washed and drained. The leaves were then blanched for 2 minutes using a dry steamer to maintain the original color of the leaves during drying.

Furthermore, the leaves were crushed with a grinder until the texture clouded. The next step was to dry the leaves using a drum dryer at a temperature of 120°C and a rotation time of 28 seconds. The dried leaves were then blended and crushed using an 80-mesh sieve. Leaves that have become flour (powder with a fine texture) can be processed. The flour was then analyzed for total flavonoids and antioxidant activity. The analysis was done at PLT UIN Syarif Hidayatullah. Observation of total flavonoids used aluminum chloride (AlCl3) based on the method of Chang et al. (2002)[37] and antioxidant activity used the DPPH (1,1-diphenyl-2-picrylhydrazyl) method from Zuhra et al. (2008)[38].

Analysis of Total Flavonoids

Analysis of total flavonoids used the aluminum chloride colorimetric test, which consisted of four stages: the first stage of sample extraction, the second stage of preparing the mother liquor, the third stage of preparing the sample solution, and the last or fourth stage of measuring the total flavonoids. The first stage of the process was that 5 grams of sample were prepared and added with 50 ml of 95% technical ethanol, then allowed to stand for 24 hours. After that, the solution was filtered and refluxed for 6 hours at 90°C. Finally, the solution was filtered and evaporated using a rotary evaporator at 45°C, 85 rpm, and a vacuum of 75 mBar.

The second stage was the preparation of the mother liquor by adding the sample extract with 1 ml of 0.5% HMTL solution, 20 ml of acetone, and 2 ml of 25% HCl solution. Then the mixture was hydrolyzed by reflux using a vertical boiler for 30 minutes. The mixture was filtered using filter paper. After being filtered, the filtrate obtained was put into a 100 ml measuring flask and then treated with acetone. Furthermore, preparing a separatory funnel, 20 ml of the filtrate results were added and shaken. Furthermore, the filtrate was added with 20 ml of distilled water and measured twice, each with 25 ml and 20 ml of ethyl acetate. After completion, the resulting ethyl acetate phase was added with ethyl acetate until the volume became 50 ml.

The third stage was the preparation of the sample solution. This stage was carried out by adding 10 ml of the mother liquor with 1 ml of 32% AlCl, then calibrated again with 5% glacial acid in methanol to 25 ml. The blank was made from a mixture of 1 ml of AlCl3 (already added 5% glacial acid in methanol to a volume of 25 ml).

The last step was to measure the total flavonoids. After adding 2% AlCl3 for 30 minutes, measurements were made with a spectrophotometer at a wavelength of 450 nm. Total flavonoids from the sample extracts tested were expressed in quercetin equivalents (Quercetin Equivalents = QE). The standard solution used was quercetin with a concentration of 0 ppm, 1 ppm, 2 ppm, 4 ppm, 8 ppm, 16 ppm, 32 ppm.

Antioxidant Activity Analysis

The first stage of the sample was weighed as much as 25 mg. Then the sample was dissolved in a 25 ml volumetric flask with methanol, and the volume was made up of methanol up to the marked line (1000 ppm mother liquor). Then, the concentration of the test solution was 4 ppm, 8 ppm, 12 ppm, and 16 ppm by taking 0.1 ml of mother liquor; 0.2 ml; 0.3 ml; and 0.4 ml into a 25 ml volumetric flask. The next step was to add 5 ml of 0.5 mM DPPH solution, the volume of which was made up of methanol up to the marked line. Then 5 ml of 0.5 mM DPPH solution was taken, put into a 25 ml volumetric flask, and filled the volume by adding methanol to the marked line for the blank solution.

The last step was to measure the absorbance of DPPH from a visible light spectrometer at a wavelength of 515 nm, with an interval of 5 minutes from 0 minutes to 30 minutes. The process of measuring antioxidant activity by looking at the decreased absorption of the DPPH solution due to additional samples was calculated as percent inhibition (% inhibition) with the following formula:

$$\% \, inhibisi = \frac{(A_{kontrol} - A_{sampel})}{A_{kontrol}} \, x \, 100\%$$

Information :
$A_{Control}$ = absorbance does not contain sample
A_{sample} = Sample absorbance

The results obtained from the calculations were entered into the regression equation with extract concentration (ppm) as the abscissa (X-axis) and the % inhibition (antioxidant) value as the ordinate (Y-axis).

Manufacturing of Torbangun Katuk Food Bars

Processing of Torbangun Katuk food bars requires tools such as ovens, mixers, bowls, spoons, knives, baking sheets, spatulas, digital scales, and baking paper. The materials needed include Torbangun flour, Katuk flour, wheat flour, eggs, margarine, full cream milk powder,

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sugar, and salt, with the composition as shown in Table 1. The Torbangun Katuk food bar was a product that has been tested organoleptically by panelists as well as proximate and physically, which has been carried out in the research of Lutfiani et al. (2021)[39]. In this study, there were three formulations of *food bar,* namely 5% (1.66% Torbangun flour and 3.34% Katuk flour), 7.5% (2.5% Torbangun flour and 5% Katuk flour), and 10% (3.34% Torbangun flour and 6.66 % Katuk flour). The formula had been subjected to comparative tests (including moisture content, ash content, protein content, fat content, and carbohydrate content), physical tests (including hardness and compactness), and organoleptic tests (color, aroma, texture, and taste) to 30 female breastfeed panelists[39]. The result of the selected formulation was the addition of Torbangun flour and Katuk flour by 7.5% with a ratio of Torbangun flour: Katuk flour 2.5%: 5%[39].

Table 1. Formulation Torbangun Katuk *food bar*

Material Name	Weight (grams)	%
Torbang flour	7	2,5
Katuk Flour	14	5
Medium protein flour Blue Triangle®	130.2	46.5
Refina® Salt	2.8	1
Gulaku® Sugar	36.4	13
Frisian Flag® full cream powdered milk	42	15
Blue Band® Margarine	14	5
Egg	33.6	12
Total	280	100

Making a food bar starts with preparing two separate containers. Margarine, eggs, powdered full-cream milk, sugar, and salt were put in the first container. The second container contains Torbangun flour, Katuk flour, and wheat flour. The ingredients in the first container were mixed with a mixer until well blended. Then the dry ingredients were mixed using a spatula in the second container until smooth. Then enter the ingredients from the second container that had been mixed into the mixture being mixed. Mix all ingredients until mixed with water, and add water gradually. The final step was to print the dough in a bar shape, and then the dough was ready to be baked in the oven at 130°C for 15 minutes. The Torbangun Katu food bar, was then analyzed for total flavonoids and their antioxidant activity using the same test method as flour.

Effectiveness Test Method

In the last stage, food bar products were tested for their effectiveness in breastfeeding mothers. The effectiveness test was carried out by giving Torbangun Katuk food bar products to breastfeeding mothers to see the success rate of the product (increasing milk production). The difference in the volume of breast milk before and after consuming a food bar was measured as a parameter of increased milk production.

At the effectiveness test stage, the research subjects were ten selected breastfeeding mothers in the Kotabaru Karawang Health Center area. The inclusion criteria in this effectiveness test were (1) Willing to be a respondent, (2) Mothers who breastfeed infants 0-6 months with exclusive breastfeeding, (3) Mothers aged 20-40 years, (4) Do not smoke and drink alcohol, (5) Mother and baby are in good health, (6) Not allergic to the ingredients used in the product. In contrast, the exclusion criteria used included (1) Mothers who could not produce breast milk, (2) Mothers with chronic diseases such as diabetes, hypertension, asthma, or breast disease problems (mastitis, breast cancer, and others), (3) Mothers taking drugs -breastfeeding drugs, both pharmacologically and herbally, and (4) mothers who receive drug therapy that has adverse side effects on milk production.

Assessment of the effectiveness of the Torbangun Katuk food bar can be seen from changes in the volume of breast milk before and after consuming the food bar. Milk volume was measured using the Dewey and Lonnerdal method using the baby's weight[40]. The KENKO Baby scale (Ministry of Health of the Republic of Indonesia 2015) was used with an accuracy of 5 grams.

The measurement procedure began with weighing the baby before and after feeding during the 24-hour measurement period. The difference in body weight before and after breastfeeding was then accumulated to obtain the baby's weight difference for 24 hours. Then the result was multiplied by 0.983 ml/g to adjust to breast milk density of breast milk40 so that the breast milk produced by the mother in one day was obtained.

Implementation of the effectiveness test began with refilling out the informed consent, and the respondent filled out the respondent's characteristic sheet. On the first day, observations were made to determine the volume of breast milk. Furthermore, the subject was given the treatment of food bars to be consumed for three consecutive days. After receiving the intervention, the subject returned to fill out the breast milk volume observation sheet to see the changes after the intervention.

Data from the effectiveness test results were then analyzed using a paired t-test. The conclusion drawn from this analysis was that if the p-value is less than 0.05, Ha was acceptable (there were differences in milk production before and after the food bar intervention). Conversely, if the p-value was more significant than 0.05, then Ha was rejected (there was no difference in milk production before and after the food bar intervention).

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RESULTS AND DISCUSSION

Product Raw Material Characteristics

One of the distinguishing factors observed in the process of making flour was yield. The yield was the weight of the flour produced compared to the weight of the processed leaves. The yield percentage was obtained by comparing the weight of flour produced with the initial weight of fresh leaves[41]. The calculation results are shown in Table 2.

Table 2. The yield of Torbangun and Katuk flour

Flour Type	Yield Value (%)
Torbang flour	5.8
Katuk flour	13.8

Chemical analysis needs to be done as a raw material for making food bars with the claim of facilitating breast milk production. The galactagogue parameters used in this study were total flavonoid content and antioxidant activity. The results of the analysis are shown in Table 3.

Table 3. The nutritional content of Torbangun flour and Katuk flour

Parameter	Torbang flour	Katuk Flour
Total Flavonoids (mg/g)	4.06	5.30
Antioxidant activity (ppm)	39.77	307.96

Based on Table 3, the total content of flavonoids in Torbangun flour was 4.06 mg/g. Compared to the leaf shape, the total flavonoid content of Torbangun flour decreased. Torbangun leaves have total flavonoids of 12.14 ± 0.42 µgQE/mg[26]. Furthermore, the study's results showed that the total flavonoids of Katuk flour were 5.3 mg/g (Table 3). Just like Torbangun flour, Katuk flour also experienced a decrease in total flavonoids from the leaf shape. Laveena and Chandra (2018) reported that the total content of Katuk leaf flavonoids was 148.94 ± 0.05 mg QE/g[34]. In both Torbangun flour and Katuk flour, there was a decrease in the total flavonoid content from the leaf form to the flour form. This decrease was due to increased temperature when processing the leaves into flour. In this process, compound decomposition occurs and affects the total flavonoid content[42].

For its function as a food ingredient for developing food bar products for breastfeeding mothers, flavonoids are phytoestrogens that can increase the diameter and number of alveolar in mammary tissue, thereby facilitating milk production[43]. It has also been confirmed that several studies state the presence of flavonoids in breast milk[44]. In previous studies, products made from Torbangun flour and Katuk flour had been shown to increase breast milk production, for example, functional food additives based on Torbangun flour[18] and lactogenic white bread with Katuk leaves[20].

In the analysis of antioxidant activity (IC50), the results were 39.77 ppm for Torbangun flour and 307.96 ppm for Katuk flour (Table 3). IC50 is the volume required to reduce 50% diphenylpicryl-hydrazyl (DPPH) radical activity[45]. The smaller the IC50 result, the stronger the antioxidant activity of the food.

Katuk flour had a higher total flavonoid content than Torbangun flour (Table 3). It is known that flavonoids have high antioxidant activity[34,46,47]. Flavonoids, as secondary plant metabolites, can function as free radical scavengers[48]. However, in this study, Katuk flour was found to have low antioxidant activity even though it had higher flavonoids. This finding is possible because only flavonoids with a specific structure in the molecule can donate protons and show radical scavenging activity[31,34]. According to toHidalgo et al. (2010), The strength of antioxidant activity depends on the nature of the radicals and the specific reaction mechanism, which is influenced by the presence of glycosidic groups, the number and position of hydroxyl and methoxy groups, and the reactions that drive structural changes. It is stated that the OH position that can provide the most potent antioxidant activity is the ortho dihydroxyl in ring B[50,51].

In the form of leaves, Katuk had the potential as an antioxidant in the moderate category because it could provide an inhibitory effect of 54.92% at a concentration of 128 ppm[25]. The decrease in antioxidant activity in Katuk flour was caused by the process of processing it into flour which involves heating. An increase in temperature and a long processing time can reduce antioxidant activity because it can damage the processed plant cell tissue and result in a changed structure of the plant[52].

In contrast to Torbangun flour, although the processing was the same as Katuk flour, its antioxidant activity was classified as very strong. Even in leaf form, the antioxidant activity of Torbangun leaves reported by Nguyen et al. (2020) was 48.23 ppm[26], which was smaller than the antioxidant activity of torbangun flour in this study (9.77 ppm) (Table 3), although both were categorized as having powerful antioxidants.

In line with research Bhave & Dasgupta (2018)**,** it was stated that the antioxidant activity of raw Torbangun leaves was lower than that of cooked Torbangun leaves (steamed for 10 minutes)[46]. This increase may be caused by damage to the rigid cell walls and a more significant release of phenolic compounds due to heating, contributing to antioxidant activity[46]. Meanwhile, Tafzi et al. (2017) mentioned that the most active compound in Torbangun with antioxidant properties was rosmarinic acid (found in the ethyl acetate and water fractions). Rosmarinic acid is a polyphenolic substance that dissolves in water and organic solvents and has a melting point of 171^{0}C-175^{0}C and a boiling point of 694.7^{0}C so that the antioxidant activity in Torbangun tends to be more stable even though it was subjected to a heating process[53].

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Determination of the Serving Size of the Test Product

The food bar product Torbangun Katuk was determined by the appropriate serving size to provide the benefits of facilitating breast milk production. The serving size was the amount of processed food consumed in one meal and was expressed in metric units and household size (URT) according to processed food[54].

The determination of serving sizes was based on Torbangun flour and Katuk flour composition, which can expedite milk production. Quoted from the research of Syarief et al. (2016), the weight of Torbangun leaves, which can increase milk production, was 120-150 grams[55]. If converted to flour, with a yield value of 5.8% (Table 2), 120-150 grams of Torbangun was equivalent to 6.96 – 8.70 grams of Torbangun flour. As for Katuk flour, it refers to research by Aminah and Purwaningsih (2013)**,** which states that giving 100 grams of Katuk increases breast milk production[56]. In grams of Katuk flour, 100 grams of Katuk is equivalent to 13.8 grams of Katuk flour (rendement score of 13.8% in Table 2).

The determination of the number of test products to be given can be seen in Table 4. The recommended portion of the Torbangun Katuk food bar is 280 grams (with seven grams of Torbangun flour and 14 grams of Katuk flour) to fulfill 100.8 mg of total flavonoids (to meet the specified baseline). The portion of the food bar was then divided into eight so that one food bar weighed 35 grams.

Table 4. Determination of the serving size of the test product

Processed products	Number of servings (grams)	Total flavonoids (mg)
Tobangung Katuk *Foodbar*	280	100.8

Product Characteristics of Torbangun Katuk Food Bar

The results of food bar products were analyzed for their nutritional content as a breast milk enhancer (galactagogue). The analysis used was the total flavonoids and antioxidant activity test. The results of the analysis of the food bars' chemical properties are presented in Table 5.

Table 5. The nutrient content of Torbangun Katuk food bars

Parameter	Results
Total Flavonoids (mg/g)	0.36±0.06
Antioxidant activity (ppm)	116.01±36.83

Table 5 shows that the Torbangun Katuk food bar had a total flavonoid of 0.36 ± 0.06 mg/g. Refers to Neshatdoust et al. (2016), a food ingredient is categorized as having high flavonoids if the total flavonoid content is >15 mg/100 g of product. In this food bar product, 100 grams of total flavonoid content is 36 mg. Therefore, products with this provision can claim to be food products with flavonoid content.

Furthermore, the antioxidant activity of Torbangun flour and Katuk flour decreased after being processed again into food bars. In Table 5, it is stated that the antioxidant activity of the food bar was equal to 116.01 ± 36.83 ppm. A small IC50 value indicates intense antioxidant activity. Antioxidants (IC50) can be classified according to their activity, namely IC50 <50 ppm including powerful antioxidants, 50-100 ppm strong, 101-150 ppm moderate, 150-200 ppm weak, and very weak if the IC50 results are more than 200 ppm[58]. The product could be categorized as food with moderate antioxidant activity from this classification.

The decrease occurred because the food bar processing involved heating, using an oven with a temperature of 130ºC for 15 minutes. Temperature and processing time affect the increase or decrease of compounds in food products, including levels and antioxidant activity. According to Narsih & Agato (2018), antioxidant activity can increase or decrease due to different temperatures and times[52].

As described in Narsih & Agato (2018), heat from processing can damage the tissues of the cooked plant cells, thereby releasing active substances in the tissues and increasing their content, but heating for too long and too high a temperature results in structural changes that deactivate the compounds in the food[52].

The Effectiveness of Torbangun Katuk Food Bar Products on Breastfeeding Mothers' Milk Production

The results of the volume of breast milk before and after giving the food bar can be seen in Table 6. Based on these results, all subjects experienced various increases in milk volume. The paired T-test was a p-value of 0.002, which means that there was a significant difference between the volume of breast milk before and after giving the food bar (p <0.05) (Table 7). Thus, giving Torbangun Katuk food bars increases breast milk volume for breastfeeding mothers.

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Table 6. Breast milk volume before and after Torbangun Katuk food bar consumption

Subject	Before giving food bar (ml)	After administration of food bar (ml)
1	216.26	516.08
2	275.24	329.31
3	176.94	452.18
4	427.61	560.31
5	289.99	491.50
6	294.90	393.20
7	127.79	339.14
8	172.03	221.18
9	196.60	270.33
10	388.29	403.03
Means	256.56±97.04	397.62±109.42

Table7. The effectiveness of treatment on the average milk production

Condition	Total Milk Production		p-value
	Means	SD	
Before treatment	256.56	97.04	0.002
After treatment	397.62	109.42	

Both Torbangun and Katuk have been known as breast milk-facilitating foods. Many studies have explained the ability of Torbangun and Katuk separately as breast milk-boosting foods. In research by Zakaria (2012)**,** it was said that combining the two will have a more significant effect than being consumed separately[32].

Table 6 shows the volume of breast milk before and after consuming the food bar. The average volume of breast milk before consuming food bars was 256.56 ml/day, and after consuming food bars, the average volume of breast milk was 397.62 ml/day. All subjects experienced varying increases in breast milk volume, so it can be concluded that all subjects experienced a significant increase in breast milk volume after consuming food bars (paired T-test $p<0.05$).

If the average volume of breast milk was compared before and after consuming food bars, there is an increase in breast milk volume of 54.98%. Studies conducted by Damanic et al. (2006) by giving Torbangun vegetables for two months show an increase in breast milk volume by 65%[40]. The results of this study were smaller than those studies due to different periods, different types of food preparations, possible differences in plant varieties, and others.

Their nutritional content influences the effect of food bars on breast milk production. Both Torbangun and Katuk are good sources of galactagogue to increase milk production. Chemical analysis carried out in this study, including total flavonoids and antioxidant activity, showed their potential as a food to increase breast milk production.

Foodbar Torbangun Katuk has a total of 100.8 mg of flavonoids. Food with a flavonoid content of >15 mg/100 g is considered high in flavonoids[57]. In one portion, which was 280 g, the food bar can contribute 100.8 mg of flavonoids (Table 4), so it can be categorized as a food high in flavonoids. Okinarum et al. (2020) stated that flavonoid compounds could provide a galactagogue effect because they have the potential to stimulate the hormones prolactin and oxytocin. Flavonoids, including phytoestrogens, are natural compounds from plants with a structure similar to estrogen. Therefore they can increase the diameter and number of alveolar in mammary tissue because it is thought to be caused by the mechanism of estrogen[43]. Thus, administering food bars containing flavonoids can increase the expression of prolactin receptor genes and glucocorticoid receptors in mammary gland epithelial cells, thereby facilitating the lactation process[29].

Antioxidants in food bar products were classified as having moderate antioxidant activity. In Table 5, it can be seen that the IC50 of the product was 116.01 ppm. Antioxidants are one of the galactagogue components that can increase milk production[60]. The composition of breast milk consists of antioxidants that prevent and protect infants from disease[61]. One can be done to increase breast milk antioxidants by consuming foods with antioxidants[29]. As a food that increases breast milk production, antioxidant activity is vital for increasing breast milk. Wirawati et al. (2017) stated that antioxidants could improve the function of alveolar cells in the mammary glands so that the epithelium is well preserved[30].

CONCLUSIONS

Torbangun and Katuk flour had the potential as additional food to increase milk production in terms of total flavonoids and their antioxidant activity. Torbangun and Katuk flour (food bars) processed products had the same manifestation. Giving Torbangun Katuk food bars from the effectiveness tests can increase milk production. In this study, there were limitations in conducting effectiveness tests. Suggestions for further research were the need to recall the mother's daily dietary intake to increase breast milk because the

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treatment given was not biased with the diet consumed by the mother. Furthermore, it was necessary to carry out direct supervision of all weighing when measuring the volume of breast milk carried out by the subject so that there was no bias in the baby's weight data and the need for effectiveness tests on a broader scale to prove the effectiveness of the Torbangun Katuk food bar.

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