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ORIGINAL ARTICLE

THE EFFECTIVENESS OF KATUK LEAF EXTRACT (*SAUROPUS ANDROGYNUS*) ON BREASTMILK PRODUCTION

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ABSTRACT

The *katuk* leaf (*Sauropus androgynus*) is believed by many Indonesian people as a traditional food that can increase breastmilk production. This leaf is rich in vitamin K, pro-vitamin A (beta-carotene), B, C, and calcium. This study investigated the effectiveness of *katuk* leaf extract on breastmilk production in experimental rabbits. The phytochemical and extraction process of the *katuk* leaves was conducted in the laboratory, USK. The experimental samples were divided into three groups: six rabbits in the control group, six in the 15 mg dose group, and six in the 30 mg dose group. An evaluation was performed by measuring the rabbit's breastmilk on days 1, 7, and 14. The ANOVA test on the results showed a p-value of 0.001 for the three groups. There was a difference in the amount of breastmilk produced between the groups, 0.04 (0.002-0.084) on the 1st day, 0.05 (0.020-0.090) on the 7th day, and 0.12 (0.065-0.175) on the 14th day. Further research can focus on investigating the *katuk* leaf extract in the clinical trial stage for its suitability in humans.

Keywords: *Breastmilk; katuk leaf extract (sauropus androgynus); milk production*

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INTRODUCTION

Breastmilk is a nutrient source that is loaded with various bioactive factors. It is needed for the optimal growth and development of babies. Infants can easily absorb and metabolize breast milk. Breastmilk can help form the infants' intestinal microbiome (Cacho & Lawrence, 2017).

The *katuk* leaf (*Sauropus androgynus*) is commonly used by breastfeeding Indonesian women to increase their breastmilk supply (Soka et al., 2010). The lactation process is stimulated by increased levels of prolactin and oxytocin hormones (H. Hayati et al., 2010). *Katuk* leaf contains almost 7% protein and up to 19% crude fiber. The leaf is rich in vitamin K, pro-vitamin A (beta-carotene), vitamin B, and vitamin C. The minerals contained are calcium (up to 2.8%), iron, potassium, phosphorus, and magnesium. The leaves are dark green due to their high chlorophyll content. *Katuk* leaves can be processed like kale or spinach. However, *katuk* leaves contain papaverine, which is an alkaloid that is also found in opium. Therefore, excessive consumption can cause side effects such as papaverine poisoning (Khooa et al., 2015).

The leaf is used in traditional medicine to treat a variety of illnesses. Additionally, it assists in weight loss and is often served as a vegetable side dish during meals. The *katuk* leaf has adequate macronutrient content, and many useful micronutrients are present. These micronutrients include minerals, carotenoids, antioxidant vitamins, and phenolic compounds. The majority of the necessary minerals, including sodium, potassium, calcium, phosphorus, iron, magnesium, copper, zinc, manganese, and cobalt, are also present in the leaf.

Katuk leaves in the Aceh Besar are known to have good durability, this is because the air temperature and weather in Aceh Besar are extreme. For that reason, it is predicted to increase breastmilk production.

Fresh *katuk* leaves typically have a moisture content of 70-90%, 3-8% protein, 1-4% fat, 1-2% fiber, and roughly 2% ash. Carbohydrates make up the remaining portion of the leaf. The types and concentrations of phenolic compounds, carotenoids, antioxidant vitamins, and minerals are discussed in this paper.

Carotenoids from *katuk* leaf extract are a source of vitamin A. Fatty acids and vitamin A, which produce retinol, interact to cause prolactin release (H. Hayati et al., 2010). When prolactin is present, the intralobular ducts' secretory glands will grow. Mammary glands can also be prepared for breastfeeding by increasing the activity of lipid-secreting and unilocular fat-containing glands (Marwah et al., 2010).

In Indonesia, it has been strongly believed for decades that the *katuk* leaf can increase breastmilk supply. Therefore, this study investigated the phytochemical content of *katuk* leaf extract and analyzed the ethanolic extract of *katuk* leaf extract on breastmilk production in experimental animals (rabbits).

METHOD

Sample

This research was conducted in three laboratories. The extraction was done at the Pharmacology Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University. *Katuk* leaf extracts were administered to experimental animals at the Experimental Animal Laboratory of Syiah Kuala University. The phytochemical process was conducted at the Research Laboratory of the Chemistry Department, Faculty of Mathematics and Natural Sciences, Syiah Kuala University.

This research was conducted from April to November 2021 on 18 female rabbits aged 4-5 months and weighing approximately 2500-3000 grams. The healthy condition of the rabbits was characterized by their active movements and not being disabled. The rabbits were kept for three months from pregnancy to birth. They were given carrots, kale, and other green vegetables every day and kept in special cages that two breeders cleaned. When the rabbits gave birth, their children remained in the same cage as them, and the researchers monitored the amount of milk the rabbits produced daily.

The extracts of young *katuk* leaves that have passed the selection process were used in this study. The leaves were taken from plants in the Indrapuri, Samahani, and Seulimum regions of the Greater Aceh District, Aceh Province, Indonesia. This is a rural area not far from the foot of the Seulawah Mountains. The topography of the Greater Aceh District consists primarily of lowland hills and a coastline between 0-1500 mdpl on the earth meridian between 5.20-5.80 north latitude and 95.00-95.80 on the equator with high rainfall and air temperatures ranging from 21℃-33℃. These conditions allow *katuk* plants to thrive and have good quality.

Instrument

The *Katuk* leaves were washed using running water and drained. After that, the dry sample was put into a drying oven and left for two days to remove the sample's water content. Next, the sample was blended into a powder, weighed, then placed into a maceration vessel soaked with 6000 mL of 96% ethanol. The maceration vessel was then closed and stored for five days away from sunlight and stirred occasionally. Furthermore, the immersion was filtered, and the macerate was evaporated using a rotary evaporator. The extraction results were then evaporated with a vacuum rotary evaporator at a temperature of 50ºC-60ºC until a thick extract was obtained.

Intervention

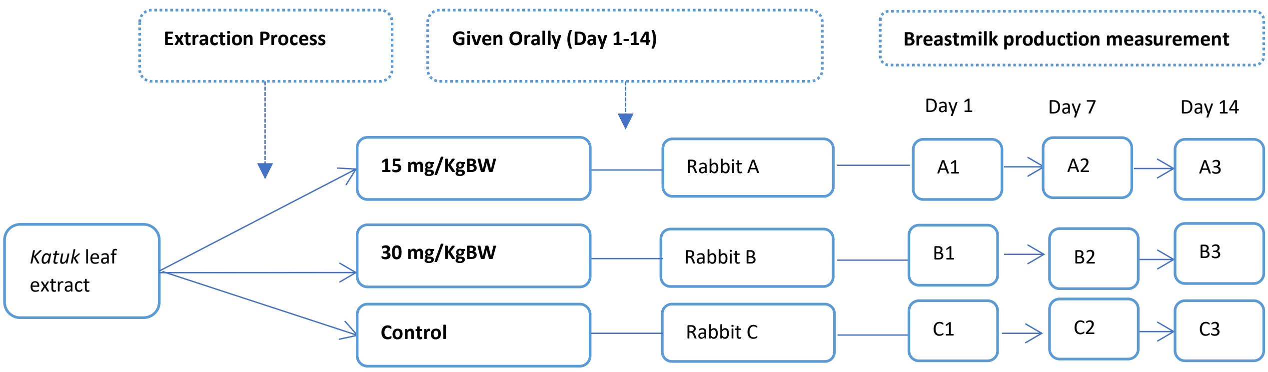
In this study, the 90% ethanol extract of *katuk* leaves underwent phytochemical screening. It was performed to provide an overview of the class of compounds contained in the plant under study. The material used for phytochemical screening was the content of alkaloid metabolites with Mayer, Wagner, and Dragendroff reagents, steroids with Liebermann-Buchard test reagents, Liebermann-Buchard test reagent terpenoids, Shaking reagent saponins, Flavonoid reagent CL, Metal Mg, Phenolic reagent FeCl1, and Tannins with Gelatin + H2SO4 reagent.

Data collection

The extract was tested on female rabbits that had just given birth (breastfeeding) by oral administration from day 1 to day 14 with the following steps: The *Katuk* leaf extract was measured per the rabbit's body weight in the intervention group at a dose of 15 mg/KgBW and 30 mg/KgBW. The dose used in this study refers to previous research conducted by Susilowati (2014). Then, the katuk leaf extract with the appropriate dose was inserted into a 3cc syringe once a day at 10 a.m. and put into the rabbit's mouth from day 1 to day 14. The amount of milk the rabbits expressed and produced was measured three times: on days 1, 7, and 14 at 5 p.m. (Scheme 1).

Data analysis

The Generalized Linear Analysis Model (Repeated ANOVA Post-hoc Bonferroni Test) was used to process the results of giving *katuk* leaf extract to three groups (two intervention groups and one control group). The same approach was applied to measure the differences in breastmilk amount between the control group and the intervention group at a dose of 15mg/KgBW, the control group with an intervention group at a dose of 30mg/KgBW, and the differences in breastmilk amount in the intervention group at a dose of 15mg/KgBW and the intervention group at a dose of 30mg/KgBW.



Scheme 1. Research design (treatment on experimental animals)

Ethical consideration
The research activity received the Ethical Clearance for Using Animals ethical permit from the Faculty of Veterinary Medicine, Universitas Syiah Kuala, with the number: 106/KEPH/V/2021.

RESULTS

Table 1 exhibits the results of the phytochemical tests on *katuk* leaf extract in experimental animals conducted at the Research Laboratory of the Chemistry Department, Faculty of Mathematics and Natural Sciences, Syiah Kuala University.

Table 1. Phytochemical test results

Metabolic Content	Reagent	Test results	Observation result
Alkaloid	Mayer	+	A white precipitate was formed
	Wagner	+	A brown precipitate was formed
	Dragendrorff	+	A red precipitate was formed
Steroid	Liebermann-Burchard test	-	No green color formed
Terpenoid	Liebermann-Burchard test	+	A red color formed
Saponin	Shuffling	-	Did not foam
Flavonoid	HCl and Mg metal	+	Changed to red
Phenolic	$FeCl_3$	+	Changed to green
Tannin	Gelatin + H_2SO_4	+	A white precipitate was formed

Note: Description (+) indicated a positive result and (-) indicated a negative result

The amount of breastmilk produced by rabbits over 14 days after giving birth was measured on days 1, 7, and 14. The amount of extract administered followed the set dose per group. Table 2 exhibits the difference in the amount of rabbit breastmilk in the control group, the intervention group at a dose of 15 mg/kg, and the intervention group at 30 mg/kg.

The Generalized Linear Model (Repeated ANOVA Post-hoc Bonferroni Test) test results show that the *p*-value for the analysis of the breastmilk amount in the three groups was 0.001. Thus, there were differences in the amount of breastmilk produced between groups in all measurements (Table 2).

Table 2. Results of differences in the amount of breastmilk in the control group, the intervention group with a dose of 15 mg/kg BW, and the intervention group with a dose of 30 mg/kgBW

Breastmilk (ml)	Control group (n = 6) Mean (SD)	Dose of 15 mg/kg BW (n = 6) Mean (SD)	Dose of 30 mg/kg BW (n = 6) Mean (SD)	p-value
Day 1	0.04 (0.03)	0.08 (0.03)	-	0.040
Day 7	0.07 (0.03)	0.13 (0.02)	-	0.005
Day 14	0.09 (0.04)	0.21 (0.04)	-	0.001
Day 1	0.04 (0.03)	-	0.06 (0.04)	0.277
Day 7	0.07 (0.03)	-	0.15 (0.03)	0.001
Day 14	0.09 (0.04)	-	0.27 (0.06)	0.001
Day 1	-	0.08 (0.03)	0.06 (0.04)	0.293
Day 7	-	0.13 (0.02)	0.15 (0.03)	0.284
Day 14	-	0.21 (0.04)	0.27 (0.06)	0.064
Day 1	0.04 (0.03)	0.08 (0.03)	0.06 (0.04)	
Day 7	0.07 (0.03)	0.13 (0.02)	0.15 (0.03)	0.001
Day 14	0.09 (0.04)	0.21 (0.04)	0.27 (0.06)	

Primary data source 2021

The results were analyzed using the Generalized Linear Model (Repeated ANOVA Post-hoc Bonferroni Test). The test found that the *p*-value for the amount of breastmilk in the control group and the group where 15 mg of *katuk* leaf extract was administered was $p < 0.05$. The confidence interval value of the mean difference of each measurement also did not exceed the number zero. Thus, there was a difference in the amount of breastmilk produced between the groups on days 1, 7, and 14 (Table 2).

Next, the Generalized Linear Model (Repeated ANOVA Post-hoc Bonferroni Test) showed that the p-value of the breastmilk amount in the control group and the group given 30mg/KgBW of the *katuk* leaf extract on the 1st day of breastfeeding measurement were $p>0.05$. The confidence interval value of the difference in the mean exceeded zero. Thus, there was no difference in the amount of breastmilk between groups on day 1. Meanwhile, on days 7 and 14, there was a difference in the amount of breastmilk in the control group and the intervention group that was administered 30 mg/Kg BW of the *katuk* leaf extract with a p-value of <0.05. The confidence interval value of the mean difference also did not exceed zero (Table 2).

Furthermore, the Generalized Linear Model (Repeated ANOVA Post-hoc Bonferroni Test) found that the p-value for the breastmilk amount in the intervention groups given 15mg/KgBW and 30mg/KgBW was $p>0.05$. The confidence interval value of the mean difference also exceeded zero. Thus, there was no difference in the amount of breastmilk produced between the intervention groups with two different doses on days 1, 7, and 14 (Table 2).

DISCUSSION

This study's result indicates that there are significant differences in the amount of breastmilk production in rabbits before and after they were administered the *katuk* leaf extract (*Sauropus androgynus*). It was proven that the intervention group given *Sauropus androgynus* (SA) extract produced more breastmilk than the control group. This finding is supported by a study conducted by Awaludin et al. (2020), which also proved that SA plants were used to increase milk production for lactating mammals because of their high sterol content. Phytosterols are plant sterols that improve progesterone synthesis and secretion and estradiol hormones. They are involved in controlling the reproduction process and the growth of mammary glands (Thambirajah et al., 2022). Another study conducted by Akbar et al. (2013) also found that the supplementation of *katuk* leaf meal increased milk production during a 3-week experiment ($p < 0.05$).

The results also showed that there was a more significant increase in breastmilk volume in the intervention group, especially at a dose of 30mg/KgBW, compared to the other two groups with a value of 0.04 (0.002-0.084) (CI 95%) on the 1st day, 0.05 (0.020)-0.090) on the 7th day, and 0.12 (0.065-0.175) on the 14th day. The analysis also revealed a difference in breastmilk volume between the intervention 30 mg/KgBW and 15 mg/KgBW intervention groups, with a difference value (CI95%) of 0.02 (-0.022-0.065) on the 1st day, 0.02 (-0.054-0.018) on the 7th day, and 0.06 (-0.121-0.004) on the 14th day. These findings align with the results of a previous study which stated that SA could increase the synthesis and production of breastmilk (Khooa et al., 2015), especially if consumed as an extract (Andarwulan et al., 2010).

The results of this study were supported by another study that conducted the experimental feeding intervention process for 12 days in two groups of lactating mice. The intervention group of mice was given SA leaf extract, and the control group was fed without SA leaves. They found that SA leaf extract supplementation increased prolactin and oxytocin gene expression in lactating mice by 9.04 and 2.25 times, respectively. Meanwhile, the administration of adult SA leaf extract inhibited the expression of both genes 15.75 and 25.77 times, respectively, compared to the control group. This was thought to be related to the papaverine content only detected in mature SA leaves (Soka et al., 2010).

In this study, the phytochemical extraction process of SA extract was conducted to ensure the quality of the resultant extract. The community has confidence in SA as a galactagogue or booster of milk production. The herbaceous plant known as *katuk* leaf (*Sauropus androgynus*) is a member of the Euphorbiaceae family. *Katuk* leaves contain nutrients and several advantageous chemicals that induce the synthesis and production of breastmilk. It enhances glucose metabolism for lactose synthesis (Nuampa & Payakkaraung, 2020).

Additionally, breastmilk production is influenced by the prolactin hormone, which plays an essential role in the lactation process. The prolactin hormone is needed to establish and maintain lactation. This hormone primarily promotes DNA synthesis, epithelial cell proliferation, milk proteins (casein, lactalbumin), free fatty acids, and lactose synthesis in the mammary glands. The level of milk protein gene transcription is stimulated explicitly by the prolactin hormone (Hall, J., 2010). Additionally, breastmilk production is strongly influenced by demand (Lactation Education Accreditation and Approval Review Committee, 2022) and the stress level of nursing mothers (Murdhiono & Okinarum, 2022).

Katuk leaves are plants with high antioxidant properties (Endrawati et al., 2022). This leaf has traditionally been used to treat certain diseases (Khooa et al., 2015) and is believed to increase breastmilk (Amalia et al., 2021; Selviana, 2022). A study by Handayani et al. (2020) found that the ideal dose of *katuk* leaf extract for increasing prolactin and oxytocin hormone expression is 900 mg/day. However, if consumed in excess in the form of fresh leaves, it can negatively impact health, such as heavy metal toxicity and lung injury (Khooa et al., 2015). Hayati et al. (2016) also stated that most traditional Indonesian communities from East Java consume *Sauropus androgynus Merr* leaves as a vegetable to increase breastmilk. The results of other studies also showed various other benefits of *katuk* leaves, such as their use as natural dyes, nutrition for goats, medicine for fever, and cough medicine (A. Hayati et al., 2016).

The limitations of this study were related to the difficulties in obtaining experimental animals that met the criteria due to the absence of special rabbit breeders. Therefore, the experimental animals were brought from other provinces, which caused the animals to undergo a long journey to the research location. Moreover, several pregnant experimental animals could not survive because of environmental disturbances that caused miscarriages.

CONCLUSION AND RECOMMENDATION

Katuk leaves contain sufficient amounts of macronutrients and essential micronutrients and minerals. It increased the breastmilk production of rabbits, as the experimental animals in this study. In this research, two doses of the *katuk* leaf extract increased breastmilk production. Between the two doses, a more significant breastmilk production was obtained from a dose of 30 mg/KgBW compared to 15 mg/KgBW. The results of this study can be used as a reference source for future clinical studies, which may be helpful for postpartum mothers. In addition, future researchers can use other experimental animals with low stress levels and check the animal's blood prolactin levels as an indicator.

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