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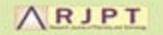
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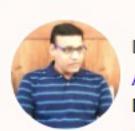
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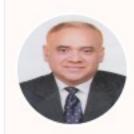
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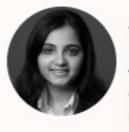
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Author(s): Shaimaa Wahman, Mohamed Emara, Riham M. Shawky

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Author(s): Priya Kurian, J. Banurekha, BS. Venkateswarlu, M. Kumar
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Author(s): Edy Parwanto, Husnun Amalia, David Tjahyadi, Hosea Jaya Edy, Ashaolu Victoria Oladimeji, Joey Joshua Vidova Tjahyadi, Laurentia Gabrielle

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Cite: Reni Anggraeni, Musheer Abdulwahid Abdo Aljaberi, Nisha Nambiar, Tukimin Bin Sansuwito, Cecep Heriana, Ruma Poddar. Pharmaceutical effect of Red Ginger (Zingiber officinale var. rubrum) on Arthritis and Gout pain in Older people at Parungkuda Public Health Center's Geriatric Polyclinic in Sukabumi Regency, Indonesia. Research Journal of Pharmacy and Technology 2023; 16(5):2115-9. doi: 10.52711/0974-360X.2023.00347

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Anti-arthritic effect of Pisonia grandis R. Br. leaves on Formaldehyde Induced Arthritis in rats

Author(s): Renuka. R, Sudha Rameshwari. K, Suguna. R, Gloria Jemmi Christobel. R, Salini. R, Latha Pandiammal. P DOI: 10.52711/0974-360X.2023.00403

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Preventive role of Dietary Phytochemical Lupeol in Preclinical Ulcerative Colitis Models

Author(s): Saumya Das, Nashra, Manas Kumar Das, Rameshwar Gaur, Daivik Mittal, Mohammad Mubashshir Shahid

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RESEARCH ARTICLE

Effect of Extreme Temperature Storage on Flavonoids levels and Antibacterial activity of *Lantana camara* Linn. leaf extract cream

Edy Parwanto^{1*}, Husnun Amalia², David Tjahyadi³, Hosea Jaya Edy⁴, Ashaolu Victoria Oladimeji⁵, Joey Joshua Vidova Tjahyadi⁶, Laurentia Gabrielle⁶ ¹Department of Biology, Faculty of Medicine, Universitas Trisakti, Indonesia. ²Department of Ophthalmology, Faculty of Medicine, Universitas Trisakti, Indonesia. ³Department of Histology, Faculty of Medicine, Universitas Trisakti, Indonesia. ⁴Study Program of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sam Ratulangi, Indonesia. ⁵Department of Chemistry, Loyola Institute of Frontier Energy, Loyola College, Chennai, India.

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ABSTRACT:

L. camara Linn. leaf extract cream has been proven to be effective as an anti-bacterial, specifically against Escherichia coli and Staphylococcus aureus. A long time storage at extreme temperature can affect its flavonoid content and antibacterial activity. Therefore, this study aims to determine the change of quercetin equivalent flavonoid levels in the L. camara Linn. leaf extract cream stored at an extreme temperature of 45 °C, and 75% relative humidity for 1 month, as well as its antibacterial activity against E. coli and S. aureus. The results showed that quercetin equivalent flavonoid levels of L. camara Linn. leaf extract cream at 3%, 4%, and 5% on day 0 are 41.76±1.03mg/100gr, 82.02±1.07mg/100gr, and 31.07±0.85mg/100gr, respectively. After storage on day 30, they were 42.43±1.14mg/100 gr, 80.51±1.24mg/100gr, and 34.34± 0.75mg/100 gr, respectively. Inhibition zone diameters of 3%, 4%, and 5% L. camara Linn. leaf extract against E. coli on day 0 were 11.52±0.71mm, 13.60±0.51mm, and 13.28±0.68mm, while after storage on day 30, they were 8.58±0.61mm, 8.58±0.62mm, and 9.08±0.23mm. Furthermore, for S. aureus on day 0, values of 16.32±0.47 mm, 13.50±0.63 mm, 13.50±0.61mm were obtained, while they were 8.52±0.76mm, 9.3±0.58mm, and 9.5±0.60mm after storage. This indicated that the quercetin equivalent flavonoid of L. camara Linn. leaf extract cream at 3%, 4% are stable after storage at 45°C and 75% relative humidity for 1 month, while it is unstable at 5%. The storage conditions for the three concentrations of L. camara Linn. leaf extract reduced the antibacterial activity against E. coli and S. aureus.

KEYWORDS: Lantana camara Linn., Flavonoid, Antibacterial cream, Escherichia coli, Staphylococcus aureus

INTRODUCTION:

Lantana camara Linn. belongs to the Verbenaceae family and is a very diverse species. Recent studies stated that it has hundreds of cultivars and hybrids, but is considered a noxious weed,¹ or an invasive plant.²

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In Indonesia, *L. camara* Linn. is known as "tembelekan", which grows wild, and is present among other plants in the Tanjakan Cino Mati area, Pleret District, Bantul Regency, Special Region of Yogyakarta.³ The plant also grows wild in various countries, but it is used as traditional medicine for treating ulcers,^{4,5} skin wounds healing,⁶ and infection.⁷ Furthermore, its leaves have been shown to have antibacterial activities.^{8,9,10}

Previous studies showed that ethanol can be used as solvent for the leaf extraction of *L. camara* Linn.^{11,12, 13}

to obtain its flavonoid content,^{12,14} tanin,^{12,13,14} alkaloid, glycoside,^{12,14} quinones,¹⁴ and anthraquinone.^{12,14} The leaf ethanolic extract also contains leuco-anthocyanins, saponosides,¹³ steroids, phenols, and coumarin.¹⁴ Furthermore, the extract contains essential oils, which are cytotoxic, including the monoterpenes hydrocarbon groups and sesquiterpenes, oxygenated monoterpenes, and sesquiterpenes.¹⁵

Escherichia coli and Staphylococcus aureus infections can cause serious global public health problems, hence, it is necessary to search for preparations to treat these two bacterial infections. This includes the use of L. camara Linn. leaf extract, which has different inhibition activities for the growth of both bacteria. The preparations containing L. camara Linn. leaf extract has strong ability to inhibit the growth of S. aureus, but weakly hinder E. coli.8 This was due to the differences in the content of active substances, including flavonoids. A previous report showed that flavonoids have antibacterial activity against E. coli and S. aureus.¹⁶ It was discovered that its levels in the L. camara Linn. leaf extract cream changed after storage for 1 year.¹⁷ Therefore, this study aims to determine the change in quercetin equivalent flavonoid levels in the L. camara Linn. leaf extract cream stored at an extreme temperature of 45°C and 75% relative humidity for 1 month (30 days), as well as its antibacterial activities for E. coli and S. aureus.

MATERIAL AND METHODS: Sample collection and extraction:

L. camara Linn. leaf was collected at Tanjakan Cino Mati, Pleret District, Bantul Regency, Yogyakarta Special Region Province. The extraction process was carried out at the Biological Laboratory, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia. The leaves were washed with water, covered with a black cloth cover, and dried in the sun. Subsequently, the dried samples were made into powder and extracted using 96% ethanol. The extract in viscous form was stored in a sterile bottle in the refrigerator and ready to be used as the active ingredient of the cream. The collection of *L. camara* Linn. leaves and its extraction were carried out in May-June 2021.

Preparation and characterization of *L. camara* Linn. leaf extract cream:

The basic ingredients of the cream are stearic acid, cetyl alcohol, liquid paraffin, methylparaben, triethanolamine, glycerol, and aquadest. Stearic acid, cetyl alcohol, and liquid paraffin were put in porcelain cup 1, while methyl paraben, triethanolamine, and glycerol were placed in cup 2. They were heated at a temperature of 70°C for the contents to melt completely without stirring. The contents were mixed in a hot mortar with rapid stirring

using a hot stemper. Aquabidestilata at a temperature of 70°C was added in a mortar and stirred continuously to form a creamy bassis. A total of 3 grams of *L. camara* Linn. leaf extract was mixed into the cream base until the volume was 100grams, to form a cream of 3%. This method was also used to make the leaf extract cream of 4% and 5%. Subsequently, organoleptic, such as shape, smell, and color,^{18,19,20} as well as pH measurement,^{3,18,20} homogeneity,^{3,19,20} and spreadability tests^{19,20,21} were carried out on the products. Preparation and characterization of the leaf extract cream was carried out in July-November 2021.

Measurement of flavonoid levels and antibacterial activity:

Measurement of flavonoid level and bacterial inhibition test was carried out at the Pharmacy Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sam Ratulangi, Manado, Indonesia, from July to November 2021. Atomic Absorption Spectrometer (AAS) was used to measure the level of flavonoids in the cream based on the standard curve that was developed before the process. Measurements of quercetin equivalent flavonoids levels were carried out on days 0, and 30. The storage of L. camara Linn. leaf extract cream was performed on the 30th day at 75% relative humidity and 45°C. Its antibacterial activity at 3%, 4%, and 5% was assessed using paper discs. The inhibition zone of E. coli as well as S. aureus was measured with an incubation time of 48 hours. The bacteria used in this study include E. coli (American Type Culture Collection/ATCC No. 1100101, USA), and S. aureus (ATCC No. 25923, Manassas, VA, USA). Changes in the level of quercetin equivalent of flavonoid were used to determine the stability of the extract. It was declared stable when changes during the storage is less than 10%.

Statistical analysis:

Differences in flavonoid level as well as antibacterial activity between the various extract concentration were tested with the one-way ANOVA. When there are differences between the groups, it is continued with the least significant difference (LSD) test. The value of P<0.05 was considered significant.

RESULTS:

Composition and characterization of *L. camara* Linn. leaf extract cream:

The composition of *L. camara* Linn. leaf extract cream is presented in Table 1. Meanwhile, its characterization, namely organoleptic tests of shape, odor, and color, as well as pH, homogeneity, and spreadability are presented in Table 2.

	Table 1. Com	position of L	camara Linn.	leaf extract cream.
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Components	L. camara Linn. leaf extract cream					
_	3 %	4 %	5 %			
Water phase						
Glycerol	8,5 mL	8,5 mL	8,5 mL			
Methyl paraben	0,2 g	0,2 g	0,2 g			
Triethanolamine	7 drops	7 drops	7 drops			
Oil phase						
Stearic acid	16 g	16 g	16 g			
Cetyl alcohol	2 g	2 g	2 g			
Liquid paraffin	10 mL	10 mL	10 mL			
L. camara Linn. leaf extract	3 g	4 g	5 g			
Aquabidest Added up to	100 g	100 g	100 g			

Abbreviation: mL = milli-liter; g = gram.

Tabel 2. The results of organoleptic testing of L. camara Linn. Leaf extract cream.

Type of cream	shape		smell		color		pН		homogeneity		spreadability	
Day of observation	H 0	H 30	H 0	H 30	H 0	H 30	H 0	H 30	H 0	H 30	H 0	H 30
Cream base	SS	SS	-	-	yw	yw	6	6	hnc	hnc	5.28 ± 0.48	5.23 ± 0.49
L. camara Linn. leaf extract cream												
3 %	SS	SS	+	+	sbg	sbg	5	5	hnc	hnc	3.22 ± 0.50	3.16 ± 0.49
4 %	SS	SS	+	+	sbg	sbg	5	5	hnc	hnc	3.13 ± 0.63	3.10 ± 0.60
5 %	SS	SS	+	+	sbg	sbg	5	5	hnc	hnc	3.17 ± 0.63	3.12 ± 0.58

Description: ss = semi solid; + = typical smell of *L. camara* Linn. leaf extract; H 0 = observation at day 0; H 30 = observation at day 30; yw = yellowish white; sbg = slightly blackish green; hnc = homogeneous not clumping.

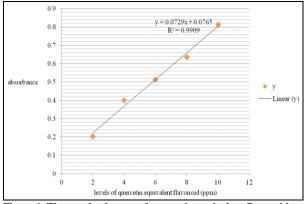


Figure 1. The standard curve of quercetin equivalent flavonoid.

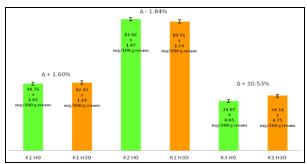


Figure 2. Level of quercetin equivalent of flavonoid in *L. camara* Linn. leaf extract cream at 3 %, 4 %, and 5 % at day 0 compared to day 30 with storage at 45 °C, and 75 % relative humidity. K1 H0 = *L. camara* Linn. leaf extract cream 3 % day 0; K1 H30 = *L. camara* Linn. leaf extract cream 3 % day 30; K2 H0 = *L. camara* Linn. leaf extract cream 4 % day 0; K2 H30 = *L. camara* Linn. leaf extract cream 4 % day 30; K3 H0 = *L. camara* Linn. leaf extract cream 5 % day 0; K3 H30 = *L. camara* Linn. leaf extract cream 5 % day 30.

The flavonoid levels of *L. camara* Linn. leaf extract cream:

The standard curve of quercetin equivalent of flavonoid is presented in Figure 1.

Level of quercetin equivalent of flavonoid in *L. camara* Linn. leaf extract cream at 3 %, 4 %, and 5 % on day 0 compared to day 30 with storage at 45 °C, and 75 % relative humidity is presented in Figure 2.

The results showed that there was no statistical difference between the level recorded at 3 % on days 0 and 30 (P = 0.288). A similar result was obtained in *L. camara* Linn. extract cream at 4 % with a p-value of 0.21. At 5 %, the quercetin level recorded on day 0 was different from day 30 (P = 0.000). It was also different when compared with 3 %, and 4 % *L. camara* Linn. leaf extract cream on both days (P = 0.000). The highest content was recorded at 4 % on days 0 and 30, followed by the 3 % concentration, while the 5 % had the lowest.

Antibacterial activity of *L. camara* Linn. Leaf Extract Cream Against *E. coli:*

Antibacterial activity of 3 %, 4 %, and 5 % *L. camara* Linn. leaf extract cream, as well as the cream base and nitrofurazone against *E. coli* on day 0, compared to day 30 with storage at 45 °C, and 75 % relative humidity is shown in Figure 3.

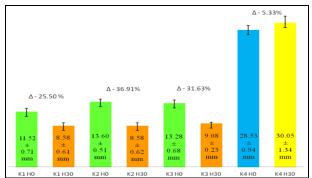


Figure 3. Inhibition zone diameter of 3 %, 4 %, and 5 % *L. camara* Linn. leaf extract cream, as well as positive control nitrofurazone against *E. coli* on day 0 compared to day 30 with storage at 45 °C, and 75 % relative humidity. K1 H0 = *L. camara* Linn. leaf extract cream at 3 % on day 0; K1 H30 = *L. camara* Linn. leaf extract cream at 3 % on day 30; K2 H0 = *L. camara* Linn. leaf extract cream at 3 % on day 0; K1 H30 = *L. camara* Linn. leaf extract cream at 3 % on day 0; K2 H30 = 4 % *L. camara* Linn. leaf extract cream on day 30; K3 H0=*L. camara* Linn. leaf extract cream at 5 % on day 0; K3 H30=*L. camara* Linn. leaf extract cream at 5 % on day 0; K4 H30=*L. camara* Linn. leaf extract cream at 5 % on day 0; K4 H30=*L. camara* Linn. leaf extract cream at 5 % on day 0; K4 H30=*L. camara* Linn. leaf extract cream of day 30; K4 H0=positive control Nitrofurazone on day 0; K4 H30 = positive control Nitrofurazone on day 30. The inhibition zone diameter of the negative control, namely cream base on days 0 and 30 is 0 mm.

On day 0, inhibition zone diameter of the extract at 3% against E. coli was different compared to the 4% and 5 % concentrations (P = 0.000), while the 4% was not different from the 5% (P = 0.479). The inhibition zone diameter of L. camara Linn. leaf extract cream at 3 % against E. coli on day 0 was different from day 30 with storage at 45°C, and 75% relative humidity (P = 0.000). Differences were also observed at 4% and 5% concentrations on both days (P = 0.000). However, there was no difference in the inhibition zone diameter of L. camara Linn. leaf extract cream at 3% and 4% against *E.* coli on day 30(P = 1.00). The result also showed that the 5% concentration on day 30 was not different from 3 % and 4% (P = 0.266). The diameter at 3%, 4%, and 5 % against E. coli on days 0 and 30 was different compared to nitrofurazone (P = 0.000). The quercetin equivalent flavonoid levels in L. camara Linn. leaf extract cream at 3%, 4%, and 5% on day 0 was different compared to day 30 after storage at 45°C, and 75% relative humidity (P = 0.000). The highest content was obtained at 4% on day 0, followed by the 3% concentration, while the lowest was recorded at 5%.

Antibacterial activity of *L. camara* Linn. leaf extract cream against *S. aureus:*

Antibacterial activity of *L. camara* Linn. leaf extract cream at 3%, 4%, 5%, as well as a cream base, and nitrofurazone against *S. aureus* on day 0 compared to day 30 after storage at 45° C, and 75% relative humidity is presented in Figure 4.

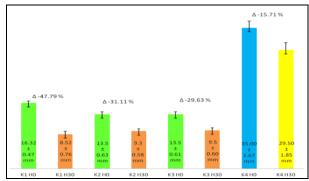


Figure 4. Inhibition zone diameter of *L. camara* Linn. leaf extract cream at 3 %, 4 %, 5 %, as well as nitrofurazone against *S. aureus* on day 0 compared to day 30. K1 H0 = *L. camara* Linn. leaf extract cream at 3 % on day 0; K1 H30 = *L. camara* Linn. leaf extract cream at 3 % on day 30; K2 H0 = *L. camara* Linn. leaf extract cream at 3 % on day 0; K2 H30 = *L. camara* Linn. leaf extract cream at 4 % on day 0; K2 H30 = *L. camara* Linn. leaf extract cream at 4 % on day 0; K2 H30 = *L. camara* Linn. leaf extract cream at 4 % on day 0; K3 H0 = *L. camara* Linn. leaf extract cream at 5 % on day 0; K3 H30 = *L. camara* Linn. leaf extract cream at 5 % on day 0; K4 H30 = Nitrofurazone on day 0; K4 H30 = Nitrofurazone on day 30. Inhibition zone diameter of cream base on both days are 0 mm.

On day 0, the inhibition zone diameter of the extract at 3 % against S. aureus was different compared to concentrations of 4% and 5% (P = 0.000). The result also showed that there was no difference between the 4 % and 5% levels (P = 1.00). Furthermore, the inhibition zone diameter of the 3% extract on day 0 compared to day 30 was different (P = 0.000). Differences were also observed at 4% and 5% on day 0 compared to day 30 (P = 0.000). There was no difference in the inhibition zone diameter of L. camara Linn. leaf extract cream at 3 % and 4 % against *E. coli* on day 30 (P = 0.195). Similar result were obtained between 3%, 4%, and 5% on day 30 (P = 0.106). The values obtained for the extract at 3 %, 4%, and 5% against E. coli on days 0 and 30 were different compared to nitrofurazone (P = 0.000). The quercetin equivalent flavonoid levels at 3%, 4%, and 5 % before storage were different from the value recorded after storage (P = 0.000). The results showed that the highest content was found at 4% on day 0, followed by 3%, while the 5% concentration level had the lowest.

DISCUSSION:

The cream base composition in this study has been optimized, as shown in Table 1. Furthermore, it was previously used to prepare *L. camara* Linn. leaf extract cream,³ and *Tagetes erecta* Linn.¹⁹ Based on the organoleptic test, the products have a semi-solid appearance, a cream-like odor, pH of 5, and the color was similar to *L. camara* Linn. leaf extract, as shown in Table 2. The test results were in line with the parameters of a quality cream. The pH obtained is normal because it is within the range of 4.5 - 6.5, and consistent with the human skin.²² Several studies revealed that some ibuprofen products have a pH range of $4.22 - 5.06.^{23}$

The cream was homogeneous, and it was characterized by the absence of lumps on the smearing result. It also had an even structure as well as a uniform color from the initial point of application to the endpoint. The tested product was collected from the top, middle, and bottom of the container. Based on the dispersion test results on days 0 and 30 above, the cream base met the requirements for topical preparations because it was within the 5-7cm spreadability range. Meanwhile, the results for the leaf extract cream at 3%, 4%, and 5% did not meet the requirements because it was less than 5 cm. These results indicate that storage at 45°C, and 75% relative humidity for 30 days did not change the spreadability of the cream base or the leaf extract by more than 10%. A similar study reported that a range of 3.76 - 3.86mm was obtained for formulation with cocoa pod peel (Theobroma cacao L.).20 The results of this study showed that the leaf extract cream at 3%, 4%, and 5% are less comfortable when used as a topical preparation on human skin. These findings are consistent with T. cacao L. cream, which was also less comfortable.

Previous studies revealed that the flavonoid content of *L. camara* Linn. leaf extract different based on the variety, and it ranges from 16.14 ± 0.21 to 25.22 ± 2.59 mg/g extract.²⁴ A previous study also demonstrated that the level of quercetin equivalent of flavonoid in the methanol extract showed high levels, namely 243.89 ± 1.30 mg/gr extract.¹⁴ Furthermore, a previous study on strychnobiflavone, which is a natural product from *Strychnos pseudoquina*, revealed that 62.5 mg/mL strychnobiflavone hydroethanolic solution contains 132.3548 mg of quercetin equivalent flavonoid and 29.770213 mg gallic acid. The results were not related to its antibacterial activity, but are associated with the free radical activity. This indicates that the study can be used as a reference.

for the importance of flavonoid content in natural products.²⁵ A previous study confirmed that the content of some methanol extract fractions of L. camara Linn. leaves collected from gardens in Wakatobi Regency, Southeast Sulawesi Province, Indonesia, ranged from 19.85±0.65-97.56±0.63mg/g sample.²⁶ For comparison, a previous study showed that the content of quercetin equivalent to flavonoid in alcohol or aqueous leaf extracts of Cucumis melo var agrestis was 30.06mg/g and 20.82mg/g, respectively.27 Previous studies have demonstrated the importance of measuring flavonoid levels as a parameter of the active ingredients in Ashwagandharishta and Amritarishta. Total flavonoids in Ashwagandharistha showed 0.013% w/w,²⁸ while in Amritarishta 0.011% w/w.²⁹ Moreover, our study's results align with the study that showed flavonoid derivatives had antibacterial activity against E. coli.³⁰

These findings indicate that there are variations in the quercetin equivalent flavonoid levels, and it is influenced by variety, environment, and solvent used for the extraction. Our statement is reinforced by the results of research which demonstrated that the extraction conditions affect the levels of flavonoids.³¹ Based on the results of our study as well as the results of other studies,²⁷ flavonoid content was measured in plant extracts^{32, 33} and herbal preparations.^{28,29,34} In addition, it has been demonstrated that the studied flavonoid derivatives are associated with antibacterial activity.³⁰

Furthermore, there was a 1.6% increase in the value obtained in L. camara Linn. leaf extract at 3% on day 30 compared to day 0, and a 1.84% decrease occurred when compared to the 4% concentration level. At 5%, a 10.53 % increase was observed on day 30 when compared to day 0. Changes were also observed at concentrations of 3%, 4%, and 5% on day 0 compared to day 30. The difference in quercetin equivalent flavonoid levels of L. camara Linn. leaf extract cream at 3%, and 4% for storage at 45°C, and 75% relative humidity for 30 days was < 10%, while an increase of 10.53% occurred at 5 %. Based on changes in the content, these results show that the extract is stable at 3%, and 4% during storage for 30 days, while it is unstable at 5%. Based on the levels of the quercetin equivalent of flavonoids, the extract was still stable at 3%, and 4% because the content was < 10 %. Several studies showed that there were changes in flavonoid levels at 3%, 4%, and 5% after storage for 1 year, namely + 85.6%, -1.07%, and +54.7%, respectively (P = 0.001).¹⁷ Meanwhile, for 120 days, the changes were + 13.54%, - 6.43%, and + 124.71% at 3%, 4%, and 5%, respectively (P = 0.001).³ Changes in flavonoid levels in this study are in line with previous studies which showed that incubation temperatures that vary, ie 20, 25, 30 and 32°C for 30 days affect the levels of flavonoids in callus culture of Heliotropium indicum Linn.35

The extract cream of L. camara Linn. at 3%, 4%, and 5 % on day 0 have strong inhibiting power against the growth of E. coli because the inhibition zone diameter was within the range of $10 - \le 20$ mm. After storage at 30°C with 75% relative humidity for 30 days, their ability to inhibit the microbe was classified as moderate with a range of $5 - \le 10$ mm. There was a 25.50% increase in the inhibition zone diameter of the extract at 3% on day 30 compared to day 0, while a decrease of 36.91% was observed at 4%. Furthermore, a 31.63% decrease occurred at 5% against E. coli on day 30 compared to day 0. The result showed that there was a huge decrease in inhibition zone diameter of the extract at 5% on day 0 compared to day 30, but no changes were observed in the positive control. These results indicate that L. camara Linn. leaf extract cream at 3%,

4%, and 5% was not stable during storage, but the positive control was stable. A previous study demonstrated that the minimal inhibitory concentration of L. camara Linn. leaf ethanolic extract collected from India against E. coli was 3mg/mL. Furthermore, at concentrations of 25mg/mL, 50mg/mL, 75mg/mL, and 100mg/mL, the inhibition zones were 4.0±0.02mm, 4.0 ±0.12mm, 3.0±0.001mm, 3.0±0.001mm, respectively.³⁶ These results are different from that of the current study. This was caused by the different varieties of L. camara Linn, which affected the levels of active ingredient in the extract. One of the ingredients is flavonoids, which play a role in inhibiting the growth of E. coli. Several studies revealed that the compound can inhibit bacterial growth by interacting with cell membranes and liposomes.³⁷ This study's results are consistent with previous studies that a solution of 5% and 10% L. camara Linn. were classified as moderate in inhibiting E. coli growth, while concentrations of 15%, 20%, and 25% were in the strong category.³⁸ Changes in flavonoid levels of the extract at 3% and 4% were not significant, but it was significant at 5%. Its formulations also experienced changes in inhibition zone diameter against E. coli. Therefore, further studies are needed to determine the flavonoid content in the extract as well as its association with temperature, humidity, and storage time.

The extract at 3%, 4%, and 5% on day 0 has strong inhibiting power on the growth of *S. aureus*, because the inhibition zone diameter was within the range of $10 - \le 20$ mm. After storage for 30 days, their ability to inhibit the microbe was classified as moderate with a range of $5 - \le 10$ mm. There was a 47.79% decrease in the diameter at 3% on day 30 compared to day 0. A 31.11% decrease also occurred at 4% against *S. aureus* on day 30 compared to day 0. There was a 29.63% reduction in the inhibition zone diameter of the leaf extract at 5%. Furthermore, a 15.71% decrease was observed in the positive control on day 0 compared to day 30. These results indicate that 3%, 4%, and 5% *L. camara* Linn. leaf extract cream and the positive control were not stable during the storage process.

Previous studies demonstrated that the extract at 5%, 10 %, 15%, 20%, and 25% concentration can strongly inhibit the growth of *S. aureus*.³⁸ Another study revealed that it contains many active substances, such as quercetin, which inhibits DNA gyrase and protein kinase, as well as disrupt bacterial cell membranes.³⁹ The condition led to membrane reduction as well as bacterial growth. The strong antibacterial activity of *L. camara* Linn. leaf extract cream in this study is consistent with several other studies.^{37,38,39} This correlation serves as a basis for its development into a phytopharmaceutical preparation. It is also important to isolate and purify the active substances, specifically the

flavonoid group. This is in line with a previous study, which showed that pectolinarin flavonoid isolated from the leaves can modulate antibacterial activity against multidrug-resistant *E. coli* and *S. aureus.*⁹ The results of our study are also in line with the results of the study who demonstrated that leaf extract of *Ipomoea aquatica* which contains flavonoids, has an antibacterial effect against *S. aureus.*⁴⁰ Moreover, our study's results also align with the study that showed flavonoid derivatives had antibacterial activity against *S. aureus.*³⁰ Eksplorasi flavonoid dari tumbuhan perlu dilakukan, hasilnya dapat digunakan sebagai bahan aktif obat terhadap *S. aureus* yang resisten berbagai jenis antibiotik.⁴¹

CONCLUSION:

L. camara Linn. leaf extract cream at a concentration of 3%, and 4% is stable based on the content of quercetin equivalent flavonoid after storage at 45°C, and 75% relative humidity for 1 month, but it is unstable at 5%. Furthermore, the storage process reduced its antibacterial activity against *E. coli* and *S. aureus*.

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ETHICAL STATEMENT:

The protocol was approved by the "Komisi Etik Riset Fakultas Kedokteran, Universitas Trisakti" (no. 034/KER/FK/IV/2022).

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CONFLICT OF INTEREST:

No conflict of interest.

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