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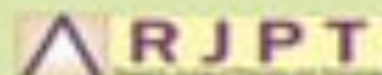
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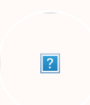
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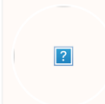
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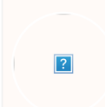
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Author(s): *Ankit Loya, Latha K, Naseeb Basha Shaik, Aisha Rahman, Javeria Tamkeen*

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In-vitro assessment of staphylococci biofilms formed under biologically-relevant conditions and correlation to the biofilm genotype

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

DOI: [10.52711/0974-360X.2023.00373](https://doi.org/10.52711/0974-360X.2023.00373)

Synthesis, Characterization and Studying of Biological Activity of some new Six-membered Compounds derived from Schiff bases

Author(s): *Nadia Sadiq Majeed, Fatima Naeem Abdul-Hussein*

DOI: [10.52711/0974-360X.2023.00374](https://doi.org/10.52711/0974-360X.2023.00374)

Evaluation of Anti-asthmatic activity of Achyranthes aspera Linn root Extract

Author(s): *Shinde Ganesh S, Rahul Kunkulol, Sandeep Narwane, Ravindra Jadhav*

DOI: [10.52711/0974-360X.2023.00375](https://doi.org/10.52711/0974-360X.2023.00375)

Antidiabetic Activity of Daun Wungu (*Graptophyllum pictum* L. Griff) Extract via Inhibition Mechanism of TNF- α , IL-6, and IL-8: Molecular Docking and Dynamic Study

Author(s): *Listijani Suhargo, Dwi Winarni, Fatimah, Viol Dhea Kharisma, Arif Nur Muhammad Ansori*

DOI: [10.52711/0974-360X.2023.00376](https://doi.org/10.52711/0974-360X.2023.00376)

Formulation and Evaluation of Buccal film of Rabeprazole sodium

Author(s): *Priya Panchal, Shobhit Srivastava, Mathew George, Nighat Anjum, Nayyar Parvez*

DOI: [10.52711/0974-360X.2023.00377](https://doi.org/10.52711/0974-360X.2023.00377)

Evaluation of Efficacy and Side Effects of Impairment Liver Function in Psoriasis Vulgaris Patients Treated with Methotrexate in Dr. Soetomo General Academic Hospital Surabaya: A Retrospective Study

Author(s): *Ira Yunita, Afif Nurul Hidayati, Muhammad Yulianto Listiawan, Evy Ervianti, Budi Utomo, Damayanti, Sylvia Anggraeni, Menul Ayu Umborowati, Cita Rosita Sigit Prakoeswa*

DOI: [10.52711/0974-360X.2023.00378](https://doi.org/10.52711/0974-360X.2023.00378)

Development and Validation of Stability Indicating Method for Simultaneous Estimation of Paritaprevir, Ombitasvir, and Ritonavir in Tablet Dosage Form

Author(s): *Naga Venkata Indira Devi Jajula, A. Krishnamanjari Pawar*

DOI: [10.52711/0974-360X.2023.00379](https://doi.org/10.52711/0974-360X.2023.00379)

Formulation and Development of Nanoparticulate System containing Rutin from Leaves Extract of *Aegle marmelos* for effective Management of Diabetes

Author(s): *Neelima Salvi, Rizwan Khan*

DOI: [10.52711/0974-360X.2023.00380](https://doi.org/10.52711/0974-360X.2023.00380)

The effect of Moringa leaves aqueous extract to Ovarian sodium dismutase and Apoptotic index in rats treated with depomedroxyprogesterone acetate

Author(s): *Ratna Dwi Jayanti, Ivon Diah Wittiarika, Rize Budi Amalia, Baksono Winardi, Sri Winarsih, I Wayan Arsana Wiyasa*

DOI: [10.52711/0974-360X.2023.00345](https://doi.org/10.52711/0974-360X.2023.00345)

Formulation and Evaluation of Polyherbal-Spirulina Based Conditioning and Antioxidant Shampoo

Author(s): *Ayyappadasan G, Rubavathi S, Kanimozli S*

DOI: [10.52711/0974-360X.2023.00381](https://doi.org/10.52711/0974-360X.2023.00381)

Microwave-Assisted Synthesis of New 4-Amino Acid Substituted 1,2,4-Triazole Derivative Derived from 1,2,3-Oxadiazole Nucleus and Their Anti-Bacterial and Anti-Oxidant Potential

Author(s): *Jaya Rautela, Ajay Singh Bisht, Vikash Jakhmola, A. N. M. Ansori, Divya Negi*

DOI: [10.52711/0974-360X.2023.00382](https://doi.org/10.52711/0974-360X.2023.00382)

RP-HPLC Method Development and Validation for Simultaneous Estimation of Rutin and Quercetin in *Morus alba* L. leaf extract

Author(s): Sarita Garg, Rubal Chahal, Deepak Kaushik, Rakesh Kumar, Vineet Mittal
DOI: [10.52711/0974-360X.2023.00383](https://doi.org/10.52711/0974-360X.2023.00383)

Development and validation of UPLC method for simultaneous estimation of Darunavir, Cobicistat, Emtricitabine and Tenofovir alafenamide in bulk drug and pharmaceutical dosage form

Author(s): Vamsi Dadi, G. Sowjanya
DOI: [10.52711/0974-360X.2023.00384](https://doi.org/10.52711/0974-360X.2023.00384)

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Author(s): Patil Pallavi M, Mayur Tekade, Samiksha Agarkar, Mohamad Taleuzzaman
DOI: [10.52711/0974-360X.2023.00385](https://doi.org/10.52711/0974-360X.2023.00385)

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Author(s): Jegarajan Pillay, Manikandan Natarajan, Siddharthan Selvaraj, Suganya Mahadeva Rao, Nirmala Devi Chandrasekaran
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Author(s): Amber Vyas, Nagendra Singh Chauhan, Tripti Jain, A.K. Singhai, Vishal Jain
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Author(s): D.B. Shirke, V. H. Karambelkar, B.S. Joshi, Chirag P. Bhattad, R. J. Jarag
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Author(s): Hadeel Saeed Hadi, Shaymaa Abdulzahra Abbas
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Author(s): Sruthy Jose, Anukrishna VP, Aarcha K Ajayan, Malavika Gopi, Rohan Rajendran, Meenu Vijayan
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Author(s): Anand Koppal, Vagdevi H. R, Senthilkumar Sivanesan, Sukumar Ethirajan, Rajagopalan Vijayaraghavan
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Author(s): *Kalesh M Karun, Deepthy M S*

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Author(s): *Peddi Srinivasa Rao, Tirukkavalluri Siva Rao, B. B. V. Sailaja, Pallapati Suman, G. Jai Sri*

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Author(s): *Mancin Stefano, Mazzoleni Beatrice*

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Author(s): *Priya Kurian, J. Banurekha, BS. Venkateswarlu, M. Kumar*

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Author(s): *Sanjay Kumar Gupta, Sradhanjali Patra*

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Author(s): *Raja Chakraverty, Chowdhury Mobaswar Hossain, Anjan Adhikari, Pranabesh Chakraborty*

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Author(s): *Yakaiah Vangoori, Naga Vishnu Kandra, Praveen Kumar Uppala, U.*

Upendrarao, S.V. Saibaba, Murali Krishna Balijepalli, Butti Lavanya, SK. M. Shabana

DOI: [10.52711/0974-360X.2023.00398](https://doi.org/10.52711/0974-360X.2023.00398)

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

Author(s): *Edy Parwanto, Husnun Amalia, David Tjahyadi, Hosea Jaya Edy, Ashaolu Victoria Oladimeji, Joey Joshua Vidova Tjahyadi, Laurentia Gabrielle*

DOI: [10.52711/0974-360X.2023.00399](https://doi.org/10.52711/0974-360X.2023.00399)

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Author(s): *Nina Nikolaevna Gavrilova, Irina Alexandrovna Ratnikova, Amankeldi Kurbanovich Sadanov, Saltanat Emilievna Orasymbet, Yerik Zharylkasynovich Shorabaev, Raushan Zhumabekovna Kaptagai*

DOI: [10.52711/0974-360X.2023.00400](https://doi.org/10.52711/0974-360X.2023.00400)

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

Author(s): *Reni Anggraeni, Musheer Abdulwahid Abdo Aljaberi, Nisha Nambiar, Tukimin Bin Sansuwito, Cecep Heriana, Ruma Poddar*

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Author(s): *Manda Pravalika, Jorige Archana*

DOI: [10.52711/0974-360X.2023.00401](https://doi.org/10.52711/0974-360X.2023.00401)

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Author(s): *S. Mary Princess Sulekha, V. Poorna Pushkala*

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Author(s): *Renuka. R, Sudha Rameshwari. K, Suguna. R, Gloria Jemmi Christobel. R, Salini. R, Latha Pandiammal. P*

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

DOI: [10.52711/0974-360X.2023.00404](https://doi.org/10.52711/0974-360X.2023.00404)

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Author(s): *Ajeet Pal Singh, Ashish Kumar Sharma, Thakur Gurjeet Singh*

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Author(s): *Paramita Das, Anjali Nayak, Bhavani K, Deepika, Ranjitha, Md Afsar*

DOI: [10.52711/0974-360X.2023.00406](https://doi.org/10.52711/0974-360X.2023.00406)

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Author(s): *Kavitha K, Srinivasan N, Suresh R, Mohan S*

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Author(s): *Shivani Sharma, Amar Deep Ankalg, Arti Devi, Mahendra Singh Ashawat*

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Author(s): *Sai Gnaneswari Aluri, Mukthinuthalapathi Mathrusri Annapurna*

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Author(s): *Zainab Fadhel Alsafar, Mohammed Sabar Al-Lami*

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Author(s): *Gazala Hussain, Vinayakumar R Kadibagil, A. Jebanesan*

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Author(s): *Mst. Mahfuza Rahman, Md. Kouser, Uthpall Kumar Roy, Shahriar Mohammad Shohan, Md. Jahirul Islam, Mst. Shagorika Shila, Sangita Chakraborty, Mir Imam Ibne Wahed*

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Author(s): *Indumathi. K. P, S. Sibyl*

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Author(s): *Megha Walia, Kajol Bhati, Jyoti Gullaiya*

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Author(s): *Hemant Mourya, Rajendra Chauhan, Ramakant Joshi, Wasim Akram, Navneet Garud*

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Author(s): *Ananya Choudhury, D. Nagasamy Venkatesh, Jey Kumar P, Mohammed Asheeq P M*

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Study Antioxidant and Antibacterial activity of Artocarpus: A Review

Author(s): *Dewi Pertiwi, Rika Hartati, Elin Julianti, Irda Fidrianny*

DOI: [10.52711/0974-360X.2023.00416](https://doi.org/10.52711/0974-360X.2023.00416)

Effect of different Processing and Preservation Techniques on Lycopene: A Mini Review

Author(s): *Shruti Rawat, Arshi Siddiqui, Rajat Singh*

DOI: [10.52711/0974-360X.2023.00417](https://doi.org/10.52711/0974-360X.2023.00417)

Novel applications of Cold Atmospheric Plasma for the treatment of Plaque Psoriasis

Author(s): *Mukesh Chandra Sharma, Mukul Sharma*

DOI: [10.52711/0974-360X.2023.00418](https://doi.org/10.52711/0974-360X.2023.00418)

Therapeutic Potential of Stingless bee Pollen: A Review

Author(s): *Annaas Budi Setyawan, U.S Mahadeva Rao, Nur Shafika Mohd Sairazi*

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Author(s): *Mukesh Sharma, Ajazuddin, Kushagra Nagori, Vishal Jain, Neema Sajju Balan*

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Development of Nanoparticles of an Antifungal Drug Incorporated in Transdermal patch

Author(s): *Kamal Kumar, Nida Parveen*

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Oral Manifestations of “COVID-19” Infection

Author(s): *Sayan Kumar Bera*

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Development and Characterization of Nanostructured Lipid Carrier for Topical delivery of Naringenin

Author(s): *Rajendra Kumar Jangde, Tanveer Khan, Harish Bhardwaj*

DOI: [10.52711/0974-360X.2023.00422](https://doi.org/10.52711/0974-360X.2023.00422)

Renoprotective effects of Guggulsterone against Cisplatin-Induced Kidney Damage in White Female Albino Rats

Author(s): *Ruya Ali Albayaty, Munaf Zalzala*

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RESEARCH ARTICLE

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

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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

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 basis. 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. Composition of *L. camara* Linn. leaf extract cream.

Components	<i>L. camara</i> 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
<i>L. camara</i> 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.

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

Type of cream	shape		smell		color		pH		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
<i>L. camara</i> 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.

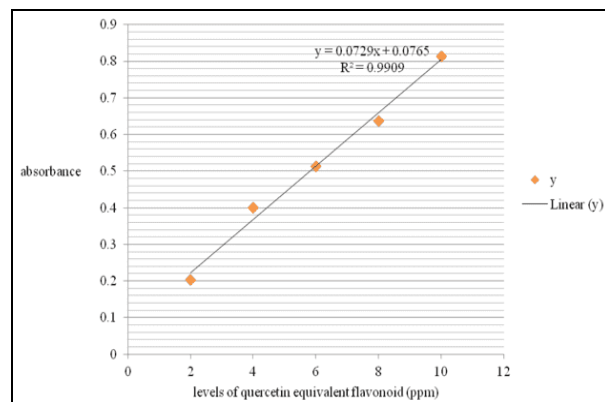


Figure 1. The standard curve of quercetin equivalent flavonoid.

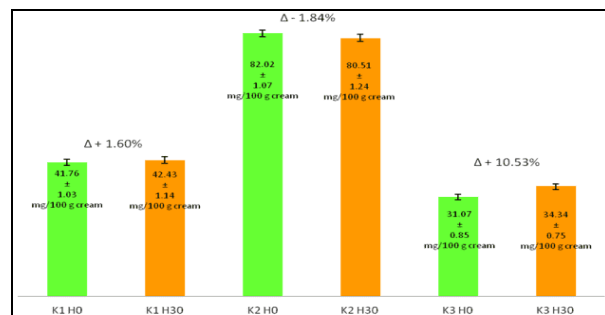


Figure 2. Level of quercetin equivalent of flavonoid in *L. camara* Linn. leaf extract cream at 3 %, 4 %, and 5 % at day 0 compared to day 30 with storage at 45 °C, and 75 % relative humidity. K1 H0 = *L. camara* Linn. leaf extract cream 3 % day 0; K1 H30 = *L. camara* Linn. leaf extract cream 3 % day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

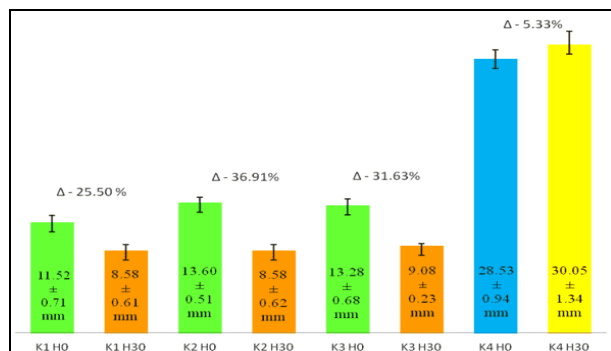


Figure 3. Inhibition zone diameter of 3 %, 4 %, and 5 % *L. camara* Linn. leaf extract cream, as well as positive control nitrofurazone against *E. coli* on day 0 compared to day 30 with storage at 45 °C, and 75 % relative humidity. K1 H0 = *L. camara* Linn. leaf extract cream at 3 % on day 0; K1 H30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

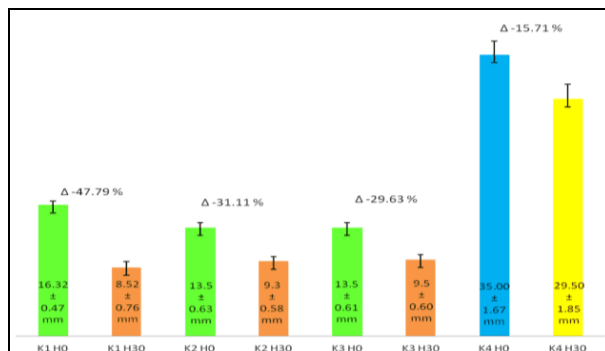


Figure 4. Inhibition zone diameter of *L. camara* Linn. leaf extract cream at 3 %, 4 %, 5 %, as well as nitrofurazone against *S. aureus* on day 0 compared to day 30. K1 H0 = *L. camara* Linn. leaf extract cream at 3 % on day 0; K1 H30 = *L. camara* Linn. leaf extract cream at 3 % on day 30; K2 H0 = *L. camara* Linn. leaf extract cream at 4 % on day 0; K2 H30 = *L. camara* Linn. leaf extract cream at 4 % 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 30; K4 H0 = 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±2.59mg/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±1.30mg/gr extract.¹⁴ Furthermore, a previous study on strychnobiflavone, which is a natural product from *Strychnos pseudoquina*, revealed that 62.5ug/mL strychnobiflavone hydroethanolic solution contains 132.3548mg of quercetin equivalent flavonoid and 29.770213mg 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 Ashwagandharishta 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 – ≤ 20mm. 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 – ≤ 10mm. 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 ± 0.02 mm, 4.0 ± 0.12 mm, 3.0 ± 0.001 mm, 3.0 ± 0.001 mm, 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 $10 - \leq 20$ mm. After storage for 30 days, their ability to inhibit the microbe was classified as moderate with a range of $5 - \leq 10$ 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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Effect of extreme temperature storage on flavonoids levels and antibacterial activity of lantana camara linn leaf extract cream

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

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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 8.12±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 *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

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}

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to obtain its flavonoid content,^{12,14} tannin,^{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 the 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 report²⁴ 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 basis. 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. Composition of *L. camara* Linn. leaf extract cream.

Components	<i>L. camara</i> Linn. leaf extract cream		
	3 %	4 %	5 %
Water phase			20
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			23
Stearic acid	16 g	16 g	16 g
Cetyl alcohol	2 g	2 g	2 g
Liquid paraffin	10 mL	10 mL	10 mL
<i>L. camara</i> 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.

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

Type of cream	shape		smell		color		pH		homogeneity		spreadability	
	H 0	H 30	H 0	H 30	H 0	H 30	H 0	H 30	H 0	H 30	H 0	H 30
Day of observation												
Cream base	ss	ss	—	—	yw	yw	6	6	hnc	hnc	5.28 ± 0.48	5.23 ± 0.49
<i>L. camara</i> 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.

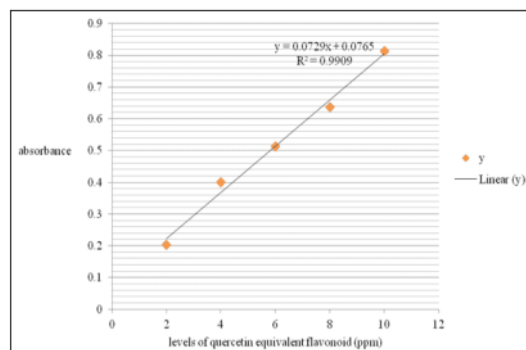


Figure 1. The standard curve of quercetin equivalent flavonoid.

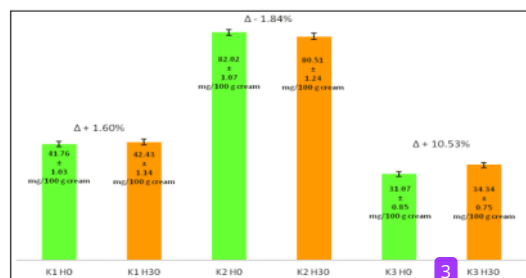


Figure 2. Level of quercetin equivalent of flavonoid in *L. camara* Linn. leaf extract cream at 3 %, 4 %, and 5 % at day 0 compared to day 30 with storage at 45 °C, and 75 % relative humidity. K1 H0 = *L. camara* Linn. leaf extract cream 3 % day 0; K1 H30 = *L. camara* Linn. leaf extract cream 3 % day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

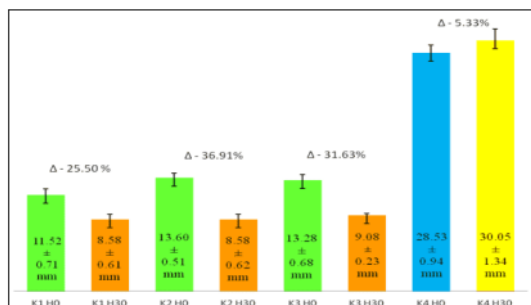


Figure 3. Inhibition zone diameter of 3 %, 4 %, and 5 % *L. camara* Linn. leaf extract cream, as well as positive control nitrofurazone against *E. coli* on day 0 compared to day 30 with storage at 45 °C, and 75 % relative humidity. K1 H0 = *L. camara* Linn. leaf extract cream at 3 % on day 0; K1 H30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

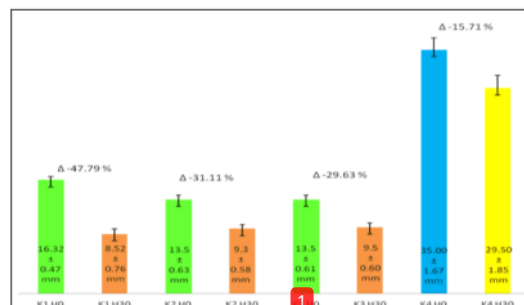


Figure 4. Inhibition zone diameter of *L. camara* Linn. leaf extract cream at 3 %, 4 %, 5 %, as well as nitrofurazone against *S. aureus* on day 0 compared to day 30. K1 H0 = *L. camara* Linn. leaf extract cream at 3 % on day 0; K1 H30 = *L. camara* Linn. leaf extract cream at 3 % on day 30; K2 H0 = *L. camara* Linn. leaf extract cream at 4 % on day 0; K2 H30 = *L. camara* Linn. leaf extract cream at 4 % 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 30; K4 H0 = 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 [9] 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±2.59mg/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±1.30mg/gr extract.¹⁴ Furthermore, a previous study on strychnobiflavone, which is a natural product from *Strychnos pseudoquina*, revealed that 62.5ug/mL strychnobiflavone hydroethanolic solution contains 132.3548mg of quercetin equivalent flavonoid and 29.770213mg 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. [19] 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 Ashwagandharishta 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 – ≤ 20mm. 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 – ≤ 10mm. 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 18.63% 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 – ≤ 20mm. After storage for 30 days, their ability to inhibit the microbe was classified as moderate with a range of 5 – ≤ 10mm. 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 pectolinarigenin 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

CONCLUSION¹³

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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CONFLICT OF INTEREST:

No conflict of interest.

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