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PREFACE

Alhamdulillah, all praise is due to Allah Ta'ala, who has bestowed the opportunity and strength so that the Scientific Journal of Pharmacy (JIF) Vol. 21 No. 1 of 2025 can be published. This issue contains ten articles, eight on the pharmaceutical science topic and two from the clinical & community pharmacy scope. The article presented in the Clinical and Community Pharmacy scope reviews the role of pharmacists in sterile compounding in hospitals and the evaluation of cost-effectiveness in cancer treatment. Meanwhile, the papers in the pharmaceutical science area include reviews and tests of drugs made from natural ingredients, their approaches using in vitro and in silico methods, and details about their formulation.

We hope that all the articles presented in this issue provide benefits and add insight to readers regarding the development of research in pharmacy and health. We eagerly await suggestions and constructive criticism from readers. We also encourage readers to contribute by submitting articles for publication in this journal. For interested readers, they can pay attention to the submission guidelines and immediately send the manuscript to our online journal system (OJS).

Finally, we wish you happy reading and apologize for any errors or omissions in the publication of this issue.

Yogyakarta, May 2025
Editor in Chief

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Physical stability test of liquid facial soap formulation of citronella extract (*Cymbopogon nardus* L.)

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Abstract

Background: Citronella (*Cymbopogon nardus* L.) is one of Indonesia's most fertile herbal plants. It contains antibacterial compounds and can be used as a liquid facial wash. With the correct formulation, citronella extract facial wash can effectively remove dirt and sebum on the face and prevent the growth of acne-causing bacteria.

Objective: This research aims to obtain the optimal formula for citronella extract liquid facial wash.

Method: Citronella extract was formulated into the liquid facial wash with concentrations of 9, 18, and 24%, and then the physical stability of the preparation was tested for 3 weeks. After that, the results are compared with the standards that have been set.

Results: The citronella extract contained saponins, polyphenols, and flavonoids. The three liquid facial wash formulations made from citronella grass extract with various concentrations had a thick, liquid texture, a unique smell, a brown color, a viscosity range of 948-2,506 cPs, a potential hydrogen range of 4.8–5.97, an adhesive test range of 1.2-2.58 seconds, a foam height range of 0-107 mm, and a spreading power of 5–6.4 cm.

Conclusion: All stability tests for citronella extract liquid facial wash formulations meet the requirements except for the foam height test. Formula 3 is the most optimal formulation, exhibiting a variance in extract concentration of 24%.

Keywords: Citronella grass, liquid facial wash, physical stability test

1. Introduction

Indonesia is situated between the continents of Asia and Australia, bordered by the Pacific Ocean and the Indian Ocean. This geographical location provides Indonesia with a distinct seasonal cycle. The variety of climates, soil types, and environmental factors contributes to Indonesia's diverse ecosystems (Setiawan *et al*., 2022). Indonesia is estimated to possess 25% of the global flora species (Kusmana & Hikmat, 2015). Indonesia's rich biodiversity encompasses 40,000 plant species, with approximately 1,300 utilized for medicinal purposes (Siregar & Ansari, 2020). Medicinal plants are commonly utilized as raw materials for pharmaceuticals or herbal remedies, which can enhance immune function upon consumption. The General Department of Plant Production defines medicinal plants as those utilized for medicinal and cosmetic purposes, derived from various plant parts including fruits, leaves, stems, and rhizomes (Siregar & Ansari, 2020). Tutik *et al*. (2022) demonstrated that the ethanol extract of citronella (*Cymbopogon nardus* L.) inhibits the growth of moderate *Staphylococcus aureus* and *Escherichia coli* bacteria. Another study by Winato *et al*. (2019) demonstrated that citronella (*C. nardus* L.) extract is capable of inhibiting the growth of *Propionibacterium acnes* bacteria. *Staphylococcus aureus*, *S. epidermidis*, and *P. acnes* are commonly implicated in acne infections.

Citronella plants possess the potential to serve as a source of raw materials for the development of essential oils. Citronella oil contains main components such as citronellal, citronellol, and geraniol (Sefriyanti *et al*., 2020). It is often used in the world's soap, perfume, cosmetics, and flavoring industries (Wijayati *et al*., 2023). In addition to its applications in these industries, citronella oil is also recognized for its natural insect-repelling properties, making it a popular choice for outdoor products and repellents. As demand for natural and sustainable ingredients continues to rise, the cultivation and processing of citronella plants present significant opportunities for growth in the essential oils market.

While the research by Rinaldi *et al*. (2021) has established the formulation of citronella ethanol extract liquid soap, this study focuses on an alternative formulation and kind of facial soap. To guarantee the product's quality, purity, and safety, conducting physical stability testing is essential. The analysis assesses the consistency of many factors, such as color, odor, and physical properties, during the storage duration (Sayuti, 2015; Allen & Ansel, 2014). This study will develop a liquid facial wash using citronella extract and assess its physical stability.

2. Method

2.1. Extraction and phytochemical screening

The citronella plants (*C. nardus* L.) were collected from Tlogowungu District, Pati Regency, Central Java. It weighed as much as 2,000 grams in a wet form that has been cleaned, dried, ground with a blender, and sieved using mesh no. 40 for homogenous simplicia powder. The extraction procedure employed the maceration method using 96% ethanol. Prior to extraction, the simplicia was measured for its moisture (water content) to ensure the dryness and avoid microorganism contamination. The phytochemical identification was conducted using several reagents to observe the color change.

2.2. Formulation of liquid facial soap loaded with citronella extract

Facial soap preparations are cleansing soaps specially formulated for healthy facial skin to safely remove oil and dirt from the surface of the facial skin (Renata & Soeyono, 2017). The formula used in this study is derived from the formulation by Yuniarsih *et al*. (2020), which creates a facial wash gel using *Hylocereus polyrhizus* extract and the gelling agent carbopol, along with an evaluation of its physical properties. In this research, citronella extract becomes the main active ingredient with various concentrations as follows: The concentration of citronella extract is 9% in the first formula (F1), 18% in the second formula (F2), and 24% in the third formula (F3).

Other materials to produce facial wash are ethylene diamine tetra acetic acid as a chelating agent, glycerin as a wetting agent, sodium lauryl sulfate as a foaming agent, propylene glycol as a solvent, nipagin as a preservative, carbopol as a gelling agent, triethanolamine as an alkali-forming agent, citric acid as a buffer-forming agent, and distilled water as a solvent. The initial step of liquid facial wash formulation involves the mixing of the solution, achieved through stirring, which consists of a blend of ethylenediamine tetra acetic acid and glycerin with distilled water. Subsequently, nipagin is incorporated, dissolved, and combined with propylene glycol. Once the mixture is deemed to be adequately formed, it is subjected to heating until it reaches a temperature of 40 degrees. Sodium lauryl sulfate was introduced gradually until a uniform mixture was achieved. Afterwards, citronella extract and citric acid were incorporated into the combination. The mixture was agitated until a uniform solution is attained. Carbopol is introduced gradually until a homogeneous mixture is achieved. Then, triethanolamine was incrementally introduced, maintaining the stirring process until a uniform mixture is attained (Yuniarsih *et al*., 2020). The formulation of liquid facial wash was then tested for its physical stability including organoleptic, viscocity, pH, foam height, adhesion, and spreadibility.

2.3. Data analysis

The SPSS software for Windows was applied in order to process the data in accordance with statistical principles. The procedure of analysis included the normality test (Shapiro-Wilk), which regarded the data to be normal if the *p*-value was more than 0.05, and the homogeneity test (Levene), which considered the data to be typical if the *p*-value was greater than 0.05: both of these tests were performed. A correlation analysis was performed on the outcomes of each test in this study to determine how well they correlated with each formula.

3. Result and discussion

3.1. Plant determination

The citronella plants (*C. nardus* L.) was determined at the Biology Laboratory of Ahmad Dahlan University with letter number 084/Lab.Bio/B/II/2024. Determination was carried out to determine the truth of the plant based on its morphological characteristics with the results as follows:

1b – 2b – 3b – 4b – 12b – 13b – 14b – 17b – 18b – 19b – 20b – 21b – 22b – 23b – 24b – 25b – 26b – 27b – 799b – 800b – 801b – 802a – 803b – 804b – 805c – 806b – 807a – 808a *Poaceae* 1b – 10b – 11b – 12b – 13b – 19a – 20a – 21b – 57b – 72b – 74b – 75b – 80a – 81b *Cymbopogon* 1b – 3b – 5a *Cymbopogon nardus* (L.) Rendle.

3.2. Citronella extract

The maceration process of citronella plant resulting an extra thick and greenish brown extract. **Table 1** displays the weight measurements of the citronella plant (*C. nardus* L.). **Table 2** shows the weight of the extract and the yield of the citronella (*C. nardus* L.) ethanol extract.

Table 1. Weight extraction simplicia citronella plant (*C. nardus* L.)

Sample	Weight before drying (g)	Weight after drying (g)
Citronella plant (*C. nardus* L.)	2000	800

Table 2. Weighting results extract and yield extracts citronella plant (*C. nardus* L.)

Sample	Weight of thick extract (g)	Yield (%)
Citronella plant (*C. nardus* L.)	53.20	10.64

A higher yield value indicates more effective and efficient extraction, as well as an increased concentration of bioactive components in the extract. In accordance with the current standard, the yield extract exceeds 10% (Kemenkes RI, 2017).

3.3. Water content test results

The moisturizer analyzer tool was used to determine the water content of citronella simplicia as shown on **Table 3** for the result. This outcome signifies that the simplicia was of high quality and adequately desiccated, as its moisture content was below 10% (Utami *et al*., 2020). Controlling the water content is important because insufficient water in the simplicia does not provide a suitable medium for microorganism growth.

Table 3. Water content test results of citronella extract (*C. nardus* L.)

Simplicia	Water content (%)
Citronella (*C. nardus* L.)	6.51

3.4. Phytochemicals screening

The phytochemical analysis of citronella extract (*C. nardus* L.) indicated the presence of flavonoids, polyphenols, and saponins, as outlined in **Table 4**. The saponin assay on the citronella plant extract produced positive outcomes, evidenced by the formation of stable foam in the extract solution. The appearance of foam is attributable to the saponin content, which is soluble in polar solvents, and the presence of chemicals that dissolve in non-polar solvents. Compounds with polar or non-polar groups exhibit surface-active properties. Saponin can form a micelle when concurrently dissolved with the solvent. A micelle structure forms as polar groups orient towards the outside. Simultaneously, the non-polar group orients towards the interior. This can be noticed as foam (Triwahyuni *et al*., 2019).

Analysis of the polyphenols in citronella extract yielded favorable results, characteristic of a blackish-green solution formation. In a polyphenol assay with $FeCl_3$ reagent, polyphenols can liberate

H^+ ions and generate phenoxy ion compounds, which subsequently interact with $FeCl_3$ to produce an iron (III) hexaphenolate complex (Rismawati *et al*., 2018).

Table 4. The result of phytochemical screening of citronella extract

Compound	Reagent	Discoloration		Conclusion
		Beginning	End	
Flavonoid	Mg + HCl powder	Brown	Brick red	Positive
Polyphenols	$FeCl_3$	Brown	Blackish green	Positive
Saponins	Aquadest	Brown	Foamy brown	Positive

3.5. Stability test of liquid facial soap with citronella extract

The physical stability of the liquid facial soap formulation was assessed over a period of three weeks, with observations conducted weekly. The tests comprised organoleptic properties, adhesion, foam height, viscosity, spreadability, and pH level. The preparation can be deemed stable if there is no substantial variation in the observed findings of the parameters each week, with a reference based on a *p*-value greater than 0.05.

3.5.1. Organoleptic test

The organoleptic testing aims to determine whether the liquid facial soap preparation meets the desired criteria. The parameters assessed in this test include odor, color, and shape. Organoleptic testing itself is carried out using the five human senses. The results of the organoleptic test of the liquid facial soap preparation with citronella extract can be seen in **Table 5**.

Table 5. Organoleptic test results of liquid facial soap with citronella extract (*C. nardus* L.)

Formulation	Parameter	Results
Basis control	The colors that emerge	Transparent white
	The emergence smell	There isn't any
	Form object	thick
F1	The colors that emerge	chocolate
	The emergence smell	Special extract
	Form object	Thick
F2	The colors that emerge	Chocolate
	The emergence smell	Special extract
	Form object	Thick
F3	The colors that emerge	Chocolate
	The emergence smell	Special extract
	Form object	Thick

The results obtained from this organoleptic test vary. The citronella extract at the basis control is colorless or transparent, viscous, and devoid of scent. The F1-F3 formulations exhibit a brown color, possess a dense texture, and emit a characteristic fragrance; as the color intensifies and becomes more concentrated, the aroma correspondingly strengthens. The intensity of the extract's color and scent increases with greater usage (Doloksaribu & Fitri, 2019). The Indonesian national standard mandates that liquid soap possess a liquid consistency, as well as a unique fragrance and

color. The results of the preparations conform to the specifications, specifically liquid consistency, unique aroma, and characteristic color of citronella extract.

3.5.2. Viscosity test results

This test aims to assess the prepared liquid face wash. The viscosity of a formula has stringent terms and conditions, and it is recommended to be neither too thick nor too liquid. The requirements for a good viscosity value range from 500 to 20,000 cPs (Gunarti, 2018). The viscosity test was performed using an assisted Brookfield viscometer with adjustments to the spindle and speed. Viscosity is strongly correlated with the concentration of the gelling agent, and in this study, carbopol was chosen since it can act as a gel base with limited safety when applied topically, as it will not trigger a reaction like hypersensitivity in the skin area (Tambunan & Sulaiman, 2018).

Table 6. Viscosity test results of citronella extract liquid facial soap *(Cymbopogon nardus* L.)

Liquid facial soap	Viscocity (week)			Mean ± SD
	1	2	3	
Basis control	1,556	948	905.3	1,136.44 ± 315.8
F1	2,427.3	1,143.3	1,098.67	1,556.44 ± 654
F2	2,234.6	2,312	1,133.3	1,893.33 ± 571.04
F3	2,506	2,410	2,354.6	2,423±70.85

Note: Viscosity meets the requirements with the value between 500-20,000 cPs (Gunarti, 2018)

Results from the viscosity test conducted over three weeks on the four formulations indicate, as presented in **Table 6**, that the facial soap preparation exhibited a reduction in viscosity value. Environmental factors in gel storage can lead to a decrease in viscosity at varying temperatures, thereby impacting the viscosity of the preparation. The viscosity measurements obtained meet the standard requirements for satisfactory preparations.

The analysis of viscosity test data indicates that the normality test yields a *p*-value greater than 0.05. The homogeneity test indicates results with $p < 0.05$, suggesting that the condition for normality is not met, as a *p*-value greater than 0.05 is required for normality. The one-way ANOVA test necessitates homogeneity of data, as indicated by Palupi & Prasetya (Palupi & Prasetya, 2022). The Kruskal-Wallis test serves as an alternative when the parametric test does not meet normality assumptions. The analysis results indicate a *p*-value of less than 0.05, suggesting significant differences in the data.

3.5.3. pH test results

The measurement of pH (hydrogen potential) seeks to evaluate the level of acidity. Products that disrupt pH equilibrium may result in skin irritation, manifesting as dryness (when pH is excessively alkaline) or redness and inflammation (when pH is excessively acidic). The potential hydrogen in liquid facial soap can markedly elevate the hydrogen potential on the skin, particularly

on the face. Consequently, it can facilitate the proliferation of bacteria that instigate the onset of acne. To avert skin irritation, the production of facial soap must establish a pH comparable to its natural acidic state, approximately 4.5 to 6.5(Marhaba *et al*., 2021).

Table 7. pH test results of citronella extract liquid facial soap (*Cymbopogon nardus* L.)

Liquid facial soap	pH (week)			Mean ± SD
	1	2	3	
Basis control	5.97	5.66	5.63	5.76 ± 0.16
F1	5.67	5.42	5.30	5.47 ± 0.16
F2	5.27	5.04	5.02	5.11 ± 0.12
F3	5.09	4.96	4.8	4.96 ± 0.12

Note: pH meets the requirements with hydrogen potential value of 4.5-6.5 (Marhaba *et al*., 2021)

According to the pH test results of the liquid facial soap formulation including citronella extract (**Table 7**) indicates that the findings conform to the hydrogen potential standards for liquid facial soap, which range from 4.5 to 6.5.

3.5.4. Foam height test results

The purpose of the foam height test is to determine the preparation's ability to produce foam. The Indonesian National Standard sets a liquid soap foam height of between 12-220 mm. According to Yuniarsih *et al*. (2020), there is no specific regulation regarding the foam height limit for facial cleansing preparations; instead, the aesthetic appeal derived from the product's foaming ability can capture consumers' attention.

Table 8. Results of the foam height test of citronella extract liquid facial soap (*Cymbopogon nardus* L.)

Liquid facial soap	Week	Beginning	End	Stability (%)
Basis control	1	0.4	0.3	74.6
	2	0.2	0.1	49.8
	3	0.2	0.1	49.8
	Average	0.26	0.17	62.6
F1	1	0.5	0.4	80
	2	0.3	0.2	66
	3	0.3	0.2	66
	Average	0.37	0.27	73
F2	1	1.3	1.2	91
	2	1.1	1	89
	3	0.6	0.5	83%
	Average	1	0.9	89%
F3	1	1.4	1.3	91.4%
	2	1.3	1.2	91%
	3	0.8	0.7	86.7%
	Average	1.17	1.07	90.2%

Note: The Indonesian national standard for height of liquid soap foam is between 12-220 mm (Maharani *et al*., 2021).

Upon analyzing the outcomes derived from the three formulations, it is evident that only F3 fulfills the criteria for an acceptable foam height. This demonstrates that an increased concentration of extract is associated with a greater foam height, given that each formula contains a consistent amount of sodium lauryl sulfate, which is incapable of emulsifying citronella extract (Maharani *et al*., 2021). In the F1 and F2, the value fails to satisfy the necessary criteria. The variation factors that may

influence foam height include the composition of materials, stirring speed, temperature, and the duration of mixing, as well as the type of active substance involved.

3.5.5. Adhesion test results

The adhesion test is carried out to determine how much the preparation can adhere to the skin within a specific time. Gel is considered good if it exhibits high adhesion. The higher the adhesion, the better, because the active substance can be absorbed evenly and diffuse maximally. The data from the adhesive power test indicated that the gel form had an influence on the adhesive power. The thicker the dosage form, the greater the adhesive power value. All results obtained meet the standard requirements of the preparation, which exceeds 1 second.

Table 9. Adhesion test of liquid facial soap with citronella extract (*Cymbopogon nardus* L.)

Liquid facial soap	Adhesion (week)			Mean ± SD
	1	2	3	
Basis control	1.77	1.66	1.2	1.55 ± 0.27
F1	2.02	1.75	1.25	1.68 ± 0.34
F2	2.15	1.85	1.32	1.78 ± 0.37
F3	2.58	2.31	1.81	2.23 ± 0.34

Note: The adhesion test meets the requirements of the adhesion time is > 1 second (Rosari *et al*., 2021)

The results of data analysis using the Kruskal-Wallis test showed results in the first week of 0.044 ($p < 0.05$), the second week showed results of 0.027 ($p < 0.05$), and the third week showed results of 0.027 ($p < 0.05$), which means that there is a difference in the data every week.

3.5.6. Spreadability test results

The spreadability test is performed to assess the distribution ability of the formulation during application, adhering to a standard of 5 to 7 cm. This characteristic is associated with viscosity; increased viscosity results in less spreadability. Increased spreadability facilitates the preparation's penetration into the skin, enhancing its efficacy (Rosari *et al.*, 2021).

Table 10. The spreadability test results of citronella extract liquid soap (*Cymbopogon nardus* L.)

Liquid facial soap	Spreadibility (week)			Average
	1	2	3	
Basis control	5.2	5.5	6.0	5.63 ± 0.35
F1	5.0	5.3	5.6	5.34 ± 0.29
F2	5.5	5.9	6.4	5.98 ± 0.38
F3	5.1	5.3	5.4	5.28 ± 0.15

Note: The spreadability test meets the requirements ranging from 5-7 cm (Rosari *et al.*, 2021).

The outcomes of the spreadability assessment for the citronella extract liquid facial soap formulation improved weekly, however it consistently remained within the appropriate spreadability range. The weekly increase may be attributed to inconsistent storage temperature and agitation, leading to a reduction in viscosity (Rosari *et al*., 2021). The findings of the data analysis employing the Kruskal-

Wallis test indicated that each week's results were < 0.05, signifying a difference in the data on a weekly basis.

4. Conclusion

The stability test of the physicochemical properties of soap formulations loaded with liquid citronella extract (*Cymbopogon nardus* L.) accross three formulations meets the following criterias: organoleptic properties, viscosity, pH value, stickiness, and spreadability. In the foam height test, only formula 3 (F3) meets the standard requirement for good foam height, which is between 12 and 220 mm.

References

Allen, L. V., & Ansel, H. C. (2014). *Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems Tenth Edition*. Philadelphia: Lippincott Williams & Wilkins.

Doloksaribu, B. E., & Fitri, K. (2019). Formulasi Sediaan Gel Hand Sanitizer Kombinasi Ekstrak Etanol Daun Kemangi (*Ocimum basilicum* L.) dan Biji Pepaya (*Carica papaya* L.). *Jurnal Dunia Farmasi*, *2*(1), 50–58. https://doi.org/10.33085/jdf.v2i1.4396

Gunarti, N. S. (2018). Pemanfaatan Ekstrak Daun Jambu Biji (*Psidium Guazava*) Sebagai Gel Facial Wash Antijerawat. *Pharma Xplore: Jurnal Ilmiah Farmasi*, *3*(2), 199–205. https://doi.org/10.36805/farmasi.v3i2.492

Kemenkes RI. (2017). *Profil Kesehatan Indonesia 2017*. Jakarta: Kementerian Kesehatan Republik Indonesia.

Kusmana, C., & Hikmat, A. (2015). The Biodiversity of Flora in Indonesia. *Journal of Natural Resources and Environmental Management*, *5*(2), 187–198. https://doi.org/10.19081/jpsl.5.2.187

Maharani, C., Suci, P. R., & Safitri, C. I. N. H. (2021). Formulasi dan Uji Mutu Fisik Ekstrak Daun Binahong (*Anredera cordifolia*(Ten.) Steenis) Sebagai Sabun Cair. *Proceeding of Mulawarman Pharmaceuticals Conferences*, *13*, 54–61.

Marhaba, F. A., Yamlean, P. V. ., & Mansauda, K. L. R. (2021). Formulasi dan Uji Efektivitas Antibakteri Sediaan Sabun Wajah Cair Ekstrak Etanol Buah Pare (*Momordica Charantia* L.) Terhadap Bakteri *Staphylococcus epidermidis*. *Pharmacon*, *10*(3), 1050–1057.

Palupi, R., & Prasetya, A. E. (2022). Pengaruh Implementasi Content Management System Terhadap Kecepatan Kinerja Menggunakan One Way Anova. *Jurnal Ilmiah Informatika*, *10*(1), 74–79. https://doi.org/10.33884/jif.v10i01.4445

Renata, G. A., & Soeyono, R. R. (2017). Survei Daya Terima Konsumen Terhadap Produk Sabun Wajah. *E-Jurnal Tata Rias. Universitas Negeri Surabaya*, *6*(1), 32–40.

Rinaldi, Elfariyanti, & Mastura, R. (2021). Formulasi Sabun Cair Dari Ekstrak Etanol Serai Wangi (*Cymbopogon nardus* L.). *Jurnal Sains Dan Kesehatan Darussalam*, *1*(1), 29–36. https://doi.org/10.56690/jskd.v1i1.10

Rismawati, Marliana, E., & Daniel. (2018). Uji Fitokimia Ekstrak Metanol Daun *Macaranga hullettii* King ex Hook.f. *Jurnal Atomik*, *3*(2), 91–94.

Rosari, V., Fitriani, N., & Prasetya, F. (2021). Optimasi Basis Gel dan Evaluasi Sediaan Gel Anti Jerawat Ekstrak Daun Sirih Hitam (*Piper betle* L. Var Nigra). *Proceeding of Mulawarman Pharmaceuticals Conferences*, 204–212.

Sayuti, N. A. (2015). Formulasi dan Uji Stabilitas Fisik Sediaan Gel Ekstrak Daun Ketepeng Cina (*Cassia alata* L.). *Jurnal Kefarmasian Indonesia*, *5*(2), 74–82.

https://doi.org/10.22435/jki.v5i2.4401.74-82

Sefriyanti, Jayuska, A., & Alimuddin, A. H. (2020). Uji Aktivitas Antibakteri Minyak Atsiri Sereh Wangi (*Cymbopogon bernadus* L .) terhadap Bakteri *Escherichia col*i dan *Staphylococcus aureus*. *Jurnal Kimia Khatulistiwa*, *8*(4), 1–4.

Setiawan, B., Hastuti, E. W., & Saleh, E. (2022). Learning Invention Using Satellite Observations to Support Sustainable Development Goals (SDG): A Use Case on Disaster Risk Reduction in Sei Serelo Indonesia. *IOP Conference Series: Earth and Environmental Science*, *1016*(1), 1–8. https://doi.org/10.1088/1755-1315/1016/1/012021

Siregar, M. Z., & Ansari, A. (2020). Penggunaan Katalis Fe terhadap Karbon Aktif Cangkang Kelapa Sawit dan Karbon Aktif Sekam Padi pada Proses Impregnasi. *Jurnal VORTEKS: Jurnal Ilmiah Teknik Mesin, Industri, Elektro, Dan Silil*, *1*(1), 13–19. https://doi.org/10.54123/vorteks.v1i1.12

Tambunan, S., & Sulaiman, T. N. S. (2018). Formulasi Gel Minyak Atsiri Sereh dengan Basis HPMC dan Karbopol. *Majalah Farmaseutik*, *14*(2), 87–95. https://doi.org/10.22146/farmaseutik.v14i2.42598

Triwahyuni, T., Rusmini, H., & Yuansah, R. (2019). Pengaruh Pemberian Senyawa Saponin dalam Ekstrak Mentimun (*Cucumissativus*) terhadap Penurunan Berat Badan Mencit (*Mus Musculus* L). *Jurnal Analisis Farmasi*, *3*(2), 59–65. https://doi.org/10.33024/jaf.v4i1.1308

Tutik, T., Chusniasih, D., & Rahayu, R. Y. (2022). Formulasi Sediaan Sabun Cair Antiseptik Ekstrak Etanol Serai Dapur (*Cymbopogon citratus* (DC.) Stapf) Terhadap Bakter *Staphylococcus aureus* dan *Escherichia coli. Jurnal Farmasi Malahayati*, *5*(1), 48–63. https://doi.org/10.33024/jfm.v5i1.6726

Utami, Y. P., Sisang, S., & Sekolah, A. B. (2020). Pengukuran Parameter Simplisia dan Ekstrak Etanol Daun Patikala (*Etlingera elatior* (Jack) R.M. Sm) asal Kabupaten Enrekang Sulawesi Selatan. *Majalah Farmasi Dan Farmakologi*, *24*(1), 5–10. https://doi.org/10.20956/mff.v24i1.9831

Wijayati, N., Pratiwi, D., Wirasti, H., & Mursiti, S. (2023). *Bab III. Minyak Serai Wangi Dan Produk Derivatnya. In Book Chapter Alam Universitas Negeri Semarang* (49–83). https://doi.org/10.15294/ka.v1i3.149

Winato, B. M., Sanjaya, E., Siregar, L., Fau, S. K. Y. M. V., & Mutia, D. M. S. (2019). Uji Aktivitas Antibakteri Ekstrak Daun Serai Wangi (*Cymbopogon nardus*) terhadap Bakteri *Propionibacterium acnes. BioLink: Jurnal Biologi Lingkungan Industri Kesehatan*, *6*(1), 50–58. https://doi.org/10.31289/biolink.v6i1.2210

Yuniarsih, N., Akbar, F., Lenterani, I., & Farhamzah. (2020). Formulasi dan Evaluasi Sifat Fisik Facial Wash Gel Ekstrak Kulit Buah Naga Merah (*Hylocereus polyrhizus*) dengan Gelling Agent Carbopol. *Pharma Xplore: Jurnal Ilmiah Farmasi*, *5*(2), 57–67. https://doi.org/10.36805/farmasi.v5i2.1194

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Integrity of *Annona muricata* L. leaves extract tablets with crospovidone

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Abstract
Background: *Annona muricata* L. (*A. muricata*) leaf contains several active compounds, including flavonoids, which are known to have antioxidant properties. People only use *A. muricata* leaves as antioxidants by boiling them in water. Therefore, the development of tablet potential is necessary to enhance user comfort and storage stability.
Objective: The aim of this research was to determine the disintegration time of *A. muricata* leaf extract tablets with varying levels of the disintegrating agent crospovidone and to determine the best disintegrating level according to the results of evaluating the physical properties of the tablets.
Methods: Tablets are made using the wet granulation method, incorporating a composition of 2-5% crospovidone. The resulting granules are tested for water content, flow time, angle of repose, and tapping. The resulting tablets were tested for hardness, friability, and disintegration time.
Results: The test results show that crospovidone and lactose can produce excellent *A. muricata* leaf extract tablets.
Conclusion: Crospovidone 3.5% produces optimum *A. muricata* leaf extract tablets with a friability of 0.36% and a disintegration time of 7.82 minutes.
Keywords: *Annona muricata* L. leaf, crospovidone, disintegrant, tablet

1. Introduction

Indonesia is home to numerous plants with medicinal properties that can be explored as traditional medicine ingredients. One such example is *Annona muricata* L. (*A. muricata*), commonly known as soursop or *sirsak*. The leaf of this plant has been traditionally used by local communities for the prevention and treatment of various diseases. Phytochemical analysis has revealed that ethanol extracts (70%) from *A. muricata* leaf contain a range of bioactive compounds, including alkaloids, flavonoids, saponins, tannins, quinone steroids, essential oils, and coumarins. These compounds exhibit antioxidant potential, which may contribute to the plant's therapeutic effects.

In vitro studies have demonstrated that *A. muricata* leaf possesses beneficial effects on pancreatic tissue function by enhancing the activity of antioxidant enzymes and insulin hormone levels. Additionally, the leaf contains acetogenin compounds with cytotoxic properties against cancer cells, suggesting a role in immune system protection and prevention of life-threatening infections (Adewole & Caxton-Martins, 2009).

In addition to containing acetogenin compounds, *A. muricata* leaf also possesses flavonoid components with potential antioxidant and therapeutic properties. Antioxidants are molecules that function by inhibiting free radical reactions in the body, protecting cells from toxic effects, and contributing to disease prevention. Traditional herbal medicines like those derived from *A. muricata* leaf tend to have fewer side effects compared to synthetic chemical-based medications. The *A. muricata* leaf extract has been shown to possess

stronger antioxidant activity than other Annona species. The various chemical compounds present in the leaf, including sesquiterpenoids, phenolic acids, and 2,3-dihydrobenzofurans, exhibit good antioxidant properties (Badmus *et al*., 2020).

A. muricata leaf is typically consumed by boiling them with water; therefore, innovative approaches are needed to facilitate their use. The leaf can be formulated into pharmaceutical preparations like tablets to enhance convenience and stability in usage. Tablet formulation requires various additives such as fillers, binders, lubricants, and disintegrants. Different formulas and processes can be combined to achieve desirable tablet properties.

One critical parameter for the success of a tablet formula is its dissolution time in the gastrointestinal tract. The addition of disintegrants like crospovidone (CPV) was performed to facilitate this process. CPV has high capillary action, allowing water to quickly enter the pores of the tablet and weaken interparticulate bonds, causing the tablet to break apart.

Prior studies have investigated and formulated tablets from *A. muricata* leaf extracts using various additives such as primojel, CMC Na - sodium starch glycolate (SSG), povidone K30 (Pov30) - croscarmellose sodium (CCS) (Endriyatno, 2018). Some studies have even specifically used the Pov30 – CPV combination. However, some of these studies did not yield tablets with a good balance between friability and dissolution time.

Therefore, this study aimed to select suitable materials supported by an appropriate compaction process. The Pov30 - CPV combination was used and re-optimized in this study. This combination theoretically will balance the tablet's integrity or strength against its dissolution rate in the gastrointestinal tract. Process parameters such as compaction were also adjusted to achieve physical properties that meet requirements.

This study is expected to provide a reference for the pharmaceutical industry in formulating herbal products, particularly *A. muricata* leaf extracts. Different formulations, even those with small differences in materials and processes, will result in different products. Therefore, this study can be used as a basis for re-optimizing or confirming previous studies' findings.

2. Method

2.1. Material

The main material used was a dried extract of *A. muricata* (Borobudur Natural Herbal Industry; ethanol extract with maltodextrin drying 10:1). The additives used were

Pov30 and CPV (Hangzhou Nanhang), lactose (Fontera), as well as magnesium stearate (Peter Greven).

2.2. Method

Tablet formulations were prepared by varying the concentration of CPV (2-5%) as shown in **Table 1**. Wet granulation was employed for tablet production. The binder was prepared by dissolving 2% Pov30 in ethanol. A mixture of dried extract, lactose, and half of the CPV was homogenized and then mixed with the binder. The wet granules were sieved, dried, re-sieved, and subsequently blended with magnesium stearate and the remaining CPV. The resulting granule mixture was subjected to evaluation. Subsequently, the granules were compressed into tablets, which were also subjected to testing (**Figure 1**).

Two analytical approaches were employed: (1) the direct method, where individual formulations were tested and compared based on physical properties, and (2) the design of experiments (DoE) approach, where a systematic mixture design was applied using Design Expert software to evaluate the effects of CPV concentration on tablet characteristics. In the direct method, each formulation was evaluated based on granule and tablet test results, with the best-performing formula selected according to critical quality attributes. The DoE approach, in contrast, utilized statistical modeling to predict the optimal composition rather than relying solely on empirical selection. This method enabled the identification of trends in formulation performance and provided a data-driven approach to optimizing tablet properties.

2.2.1. Granule evaluation

The granule flow test was conducted after mixing, prior to compression into tablets. The powder flow test was performed using a funnel and tapping. The flow rate was determined by pouring 100 g of powder through the funnel and recording the time taken for it to pass through. The flowed powder was then measured for its height and diameter to obtain an angle of repose value (Awaluddin *et al*., 2017; USP, 2016).

Table 1. Formula of *A. muricata* leaf extract tablets

Material (mg)	FI	FII	FIII
A. muricata Leaf Extract	100 mg	100 mg	100 mg
Lactose	370 mg	362.5 mg	355 mg
CPV	10 mg	17.5 mg	25 mg
Pov30 2%	10 mg	10 mg	10 mg
Mg stearate 2%	10 mg	10 mg	10 mg

Figure 1. Flowchart of the preparation and testing of *A. muricata* leaf extract tablets

The tapping test was conducted on 100 mL of powder, which was tapped a total of 100 times. This process continued until a constant volume was achieved. The tapping index was determined based on the calculation between the final and initial volumes (100 mL) (USP, 2016).

2.2.2. Tablet evaluation

The tablet testing was conducted on tablets after compression, which included tests for weight, hardness, disintegration, and friability (USP, 2016). The weight test was performed by weighing ten tablets on an analytical balance to determine any variation in weight between tablets. The hardness of the tablets was evaluated using a hardness testing device. The hardness value was determined as the mean of 10 measurements.

The next test was conducted by placing ten tablets in a friability tester. The tablet was rotated on this device for four minutes at a speed of 25 RPM. Subsequently, the disintegration test was performed with six samples of each formula, which were placed in an aqueous medium. The results of the test were determined based on the duration until the tablets had completely disintegrated and no granule residue remained on the mesh surface (Martinello *et al*., 2006).

3. Results and discussion

3.1. Granule evaluation

The granule evaluation was conducted on dried granules to determine whether their characteristics meet the requirements for good tablet quality, as the properties of the granules will affect the quality of the tablets (**Figure 2**). The results of the granule evaluation can be seen in **Table 2**.

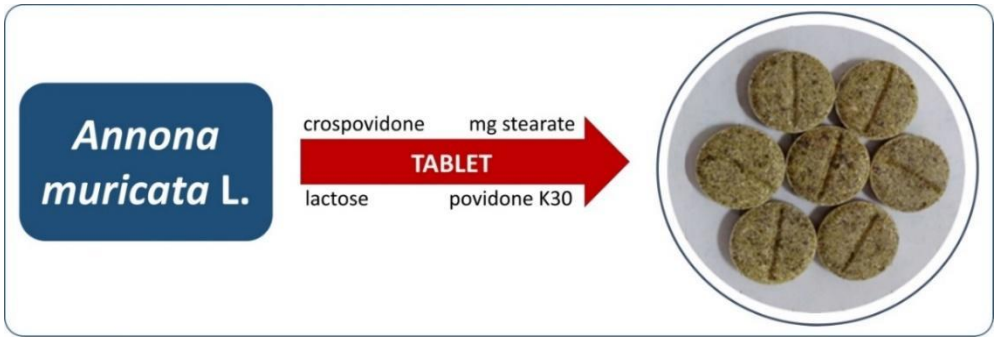

Figure 2. Illustration of tablet formulation
Note: The image used is Tablet F2. Other formulas have similar-looking tablets.

Table 2. Granule properties of *A. muricata* leaf extract tablets

Parameter	F1	F2	F3
Moisture content (%)	2.12±0.23	1.48±0.29	1.81±0.46
Flow speed (g/s)	14.23±0.05	15.02±0.18	14.39±0.06
Angle of repose (°)	30.53±1.11	30.52±1.11	30.67±0.96
Tapping index (%)	20.00±0.81	22.66±1.52	23.00±1.00

3.1.1. Moisture content

The moisture content test on granules was conducted using a moisture balance at 105°C. The level of moisture will affect the manufacturing and storage processes of the dosage form. Factors that influence moisture content include room humidity, drying of granules, and physical properties of the extract itself. This test is very important because it relates to flowability, compaction process, compactibility, and stability. In general, the moisture content of granules ranges from 2-5%. The results show that all three formulas have achieved a suitable moisture level, ranging from 1.48 to 2.12%. The heating performed for 24 hours in an oven played a significant role in achieving this result. Optimal moisture levels will affect the compaction process of tablets. Granules that are too moist will make tablets very hard and slow down their disintegration time. On the other hand, extremely dry granules will make tablets prone to being fragile (Gabbott *et al*., 2016; USP, 2016).

3.1.2. Flow speed

The United States Pharmacopeia (USP) recommends evaluating granule flow rate as a parameter to assess the smoothness of granules flowing into tablet compression machines. The quality of this flow is influenced by several factors. including particle size, shape, and density, as well as moisture content. Poor granule flow can result in inconsistent tablet weights, compromising the uniform distribution of active ingredients (Goh *et al*., 2017).

The test results show that all formulas have a good flow rate, namely > 10 g/second. The binder Pov30 can help in maintaining cohesion between particles so that powder from different materials can remain united as one compact granule. Since the granules are

physically larger than powders, their flow rates will be even better (Lamešić *et al*., 2017; USP, 2016).

3.1.3. Angle of repose

The angle of repose is commonly utilized to determine granule flow characteristics. The angle of repose refers to the maximum angle formed between the surface of a material and a flat plane when that material is poured from a funnel. The angle of repose can provide information about the smoothness of flow and the ability of a material to flow. The smaller the angle of repose, the better the flow properties of the material are. Test results show that all formulas have good angles of repose because they are <35°. The differences in variations among the three formulas do not result in significant differences in the angle of repose values. Instead, the testing results indicate that any formula used in this study will produce powders with good flow properties (Kaleemullah *et al*., 2017; USP, 2016).

3.1.4. Tapping evaluation

The tapping test is an indirect method for evaluating granule flow properties by assessing the material's ability to settle after repeated vibrations. The test is quantified using the T% index (%), where lower values indicate better settling and improved flowability, typically associated with fewer fines capable of filling interparticle spaces. The T% index is largely influenced by the granule mixture's ability to fill voids and achieve tighter packing during vibration. Flowability is determined by multiple factors. including particle shape, brittleness, and size distribution. A T% index of less than 20% is generally indicative of good flow properties, with smaller T% values reflecting superior flowability (Alkrad *et al*., 2017; USP, 2016).

The calculated values from Formulas I, II, and III yielded results of 20%, 22.66%, and 23% respectively. Based on these values, it can be concluded that the granule exhibits moderate flow properties due to its T-index falling within the range of 20-23%. This suggests that the granules have an irregular shape, a non-uniform size distribution, were exposed to air for too long, and experienced significant volume reduction, ultimately resulting in a high T-index value (Alkrad *et al*., 2017; USP, 2016).

3.2. Tablet evaluation

3.2.1. Weight variation

The weight variation test, as described in **Table 3**, is a method for determining the level of weight variability within a batch production. This testing ensures that each tablet in a single batch has a uniform or only slightly deviating weight from the average weight. Insufficient weight can result in a dose lower than specified, leading to reduced efficacy.

Conversely, excessive weight may cause an overdose of active ingredients, increasing the risk of adverse effects and exceeding specifications (Kaleemullah *et al*., 2017; USP, 2016).

Table 3. Evaluation results of the physical properties of *A. muricata* leaf extract tablets

Evaluation	F1	F2	F3
CV of weight (%)	1.05	2.18	1.98
Hardness (kgf)	5.37±0.42	5.31±0.30	5.40±0.68
Friability (%)	0.16±0.22	0.36±0.30	0.55±0.70
Disintegration time (min)	13.98±3.77	7.82±0.10	5.68±0.07

3.2.2. Tablet hardness

The hardness test is conducted to determine the level of firmness or strength of a tablet. This testing ensures that the produced tablets have an appropriate hardness, preventing them from breaking easily during production, distribution, and storage processes. Additionally, it guarantees that the tablets can be quickly disintegrated with ease in the body upon consumption (Martinello *et al*., 2006; USP, 2016).

3.2.3. Tablet friability

The friability test is conducted to determine a tablet's resistance to impact and vibration. This testing ensures that the produced tablets have sufficient mechanical strength, preventing them from breaking or becoming damaged during processes such as compression, packaging, and transportation. This test is crucial because high friability can cause damage to the shape of pharmaceutical preparations, ultimately reducing their efficacy (Alkrad *et al*., 2017; USP, 2016).

3.2.4. Disintegration test

The disintegration test assesses how rapidly a tablet breaks down into small particles and releases its active compounds within bodily fluids. This testing employs water as the medium, simulating the conditions of gastric or intestinal fluid erosion using an apparatus that moves up and down. For immediate-release tablets, it is anticipated that they will quickly disintegrate upon contact with these fluids, thereby delivering the intended therapeutic effect in a timely manner (Martinello *et al*., 2006).

In this formulation, crospovidone (CPV) as super disintegrant can absorb water and swell to push out particle matrix of tablets. This will accelerate tablet disintegration when it comes into contact with gastrointestinal fluids. The longest disintegration time was achieved by F1 with the lowest grinding material content, while the fastest was F3 with the highest CPV composition. All formulas showed good results for disintegration times. Therefore, at least, these tablets can quickly disintegrate within 15 minutes when in contact with gastrointestinal fluids. However, selecting the best formula cannot solely be based on the fastest disintegration time. Other parameters such as tablet friability must also be considered to determine the optimal formulation (Awaluddin *et al*., 2017; USP, 2016).

3.3. General testing and optimal formulation

The results from the direct method showed that F2 exhibited the best overall performance, with a friability of 0.36% and a disintegration time of 7.82 minutes. In contrast, F1 had lower friability but a significantly longer disintegration time, nearing 15 minutes. Meanwhile, F3 demonstrated a shorter disintegration time but higher friability. Notably, in one measurement, F3 exceeded the standard friability limit of 1%, reaching 1.35%, resulting in a high standard deviation. Based on these findings, F2 was identified as the most favorable formulation among the tested samples.

In the second approach, the design of experiments (DoE) method, an optimization simulation was performed using a mixture design in Design Expert software (**Figure 3**). The goal was to minimize both friability and disintegration time while identifying the optimal composition. The predicted optimal formulation consisted of LAK:CPV at a ratio of 363.13 mg:16.86 mg, corresponding to a CPV concentration of 3.4%. The model predicted a disintegration time of 9.51 minutes and a friability of 0.34%. While the predicted disintegration time was slightly longer than that of F2, the lower friability suggested improved mechanical integrity. This approach allowed for a systematic evaluation of formulation variables, demonstrating that statistical modeling could predict an optimized composition beyond simply selecting the best-performing tested sample.

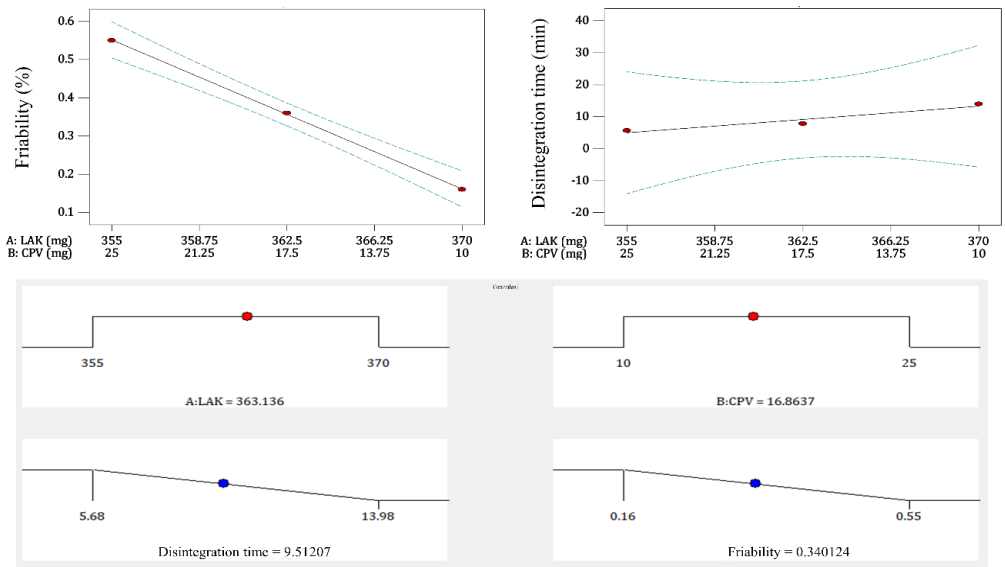

Figure 3. Formula optimization using mixture design

The variability observed in tablet friability testing across measurements is likely attributed to the mixing process. Prolonged mixing or optimizing the mixing speed may address this issue. However, it is important to note that the variations do not overlap and

still exhibit a consistent trend in friability percentages: F1 < F2 < F3. Thus, these inconsistencies do not alter the conclusions regarding tablet friability.

Another parameter to consider is compression pressure. All tablet formulations were produced under the same compression pressure, resulting in tablet hardness of approximately 5 kgf. Slightly increasing the compression force could further reduce the friability values. Nonetheless, even under the current parameters, all formulations generally meet the requirements for tablet friability testing (Alkrad *et al*., 2017).

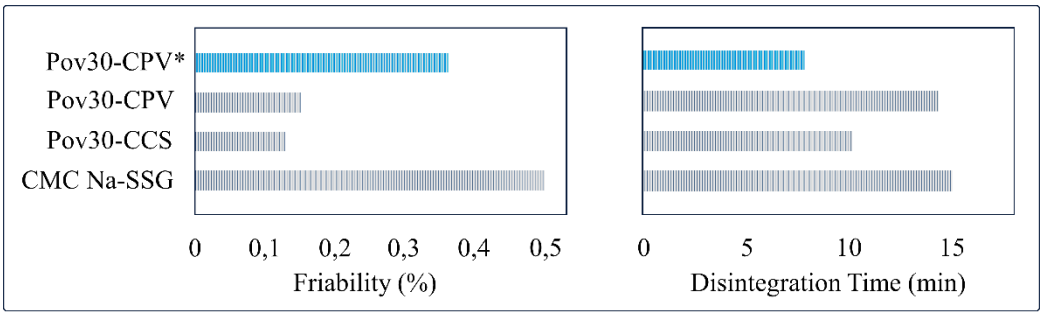

Figure 4. Comparison of *A. muricata* leaf extract tablet formulations in this study (*) with other studies using different excipients

One of the primary testing parameters for herbal tablets is disintegration time, which indicates how quickly a tablet breaks apart and releases its active ingredient upon contact with gastrointestinal fluids. F2, identified as the optimal formulation, exhibited excellent disintegration performance, with a value of under 10 minutes. Compared to formulations reported in other studies (**Figure 4**), which utilized disintegrants such as povidone K30-CPV, povidone K30-CCS, and CMC-Na – SSG, the F2 formulation demonstrated superior disintegration performance. While some of these alternative excipient systems may have resulted in lower friability, their disintegration times were generally longer. The rapid disintegration of F2 suggests that the specific ratio of Pov30 - CPV in this study provided a more efficient tablet breakdown, which is a critical factor for herbal tablet formulations (Endriyatno, 2018).

4. Conclusion

A. muricata was successfully formulated into tablets using povidone K30 as a binder and CPV as a super disintegrant. The direct method identified Formula II (containing 3.5% CPV) as the best-performing formulation, exhibiting a friability of 0.36% and a disintegration time of 7.82 minutes. Meanwhile, the design of experiments (DoE) approach predicted an optimized formulation with 3.4% CPV, yielding a friability of 0.34% and a disintegration time of 9.51 minutes. These findings demonstrate that statistical modeling

can provide an optimized formulation beyond empirical selection, balancing key tablet properties for enhanced performance.

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References

Adewole, S., & Caxton-Martins, E. (2009). Morphological Changes and Hypoglycemic Effects of *Annona muricata* Linn. (Annonaceae) Leaf Aqueous Extract on Pancreatic B-Cells of Streptozotocin-Treated Diabetic Rats. *African Journal of Biomedical Research*, *9*(3), 173–187. https://doi.org/10.4314/ajbr.v9i3.48903

Alkrad, J. A., Abu Shmeis, R., Alshwabkeh, I., Abazid, H., & Mohammad, M. A. (2017). Investigation of The Potential Application of Sodium Bentonite As An Excipient In Formulation of Sustained Release Tablets. *Asian Journal of Pharmaceutical Sciences*, *12*(3), 259–265. https://doi.org/10.1016/j.ajps.2017.01.004

Awaluddin, R., Prasetya, A. W., Nugraha, Y., Suweleh, M. F., Kusuma, A. P., & Indrati, O. (2017). Physical Modification and Characterization of Starch Using Pregelatinization and Co-Process of Various Tubers From Yogyakarta As An Excipient. *AIP Conference Proceedings*, *1823*(March 2017), 1–10. https://doi.org/10.1063/1.4978184

Badmus, J. A., Oyemomi, S. A., Adedosu, O. T., Yekeen, T. A., Azeez, M. A., Adebayo, E. A., Lateef, A., Badeggi, U. M., Botha, S., Hussein, A. A., & Marnewick, J. L. (2020). Photo-Assisted Bio-Fabrication of Silver Nanoparticles Using *Annona muricata* Leaf Extract: Exploring The Antioxidant, Anti-Diabetic, Antimicrobial, and Cytotoxic Activities. *Heliyon*, *6*(11), 1–9. https://doi.org/10.1016/j.heliyon.2020.e05413

Endriyatno, N. C. (2018). *Optimasi Formula Tablet Ekstrak Daun Sirsak (Annona muricata L.) Dengan Bahan Pengikat Cmc Na dan Penghancur Explotab Menggunakan Metode Factorial Design*. Skripsi. Program Studi Farmasi Fakultas Farmasi Universitas Muhammadiyah Surakarta.

Gabbott, I. P., Al Husban, F., & Reynolds, G. K. (2016). The Combined Effect of Wet Granulation Process Parameters and Dried Granule Moisture Content On Tablet Quality Attributes. *European Journal of Pharmaceutics and Biopharmaceutics*, *106*, 1–19. https://doi.org/10.1016/j.ejpb.2016.03.022

Goh, H. P., Heng, P. W. S., & Liew, C. V. (2017). Understanding Die Fill Variation During Mini-Tablet Production. *International Journal of Pharmaceutics*, *534*(1–2), 1–34. https://doi.org/10.1016/j.ijpharm.2017.10.042

Kaleemullah, M., Jiyauddin, K., Thiban, E., Rasha, S., Al-Dhalli, S., Budiasih, S., Gamal, O. E., Fadli, A., & Eddy, Y. (2017). Development and Evaluation of Ketoprofen Sustained Release Matrix Tablet Using Hibiscus Rosa-Sinensis Leaves Mucilage. *Saudi Pharmaceutical Journal*, *25*(5), 770–779. https://doi.org/10.1016/j.jsps.2016.10.006

Lamešić, D., Planinšek, O., Lavrič, Z., & Ilić, I. (2017). Spherical Agglomerates of Lactose With Enhanced Mechanical Properties. *International Journal of Pharmaceutics*, *516*(1–2), 1–38. https://doi.org/10.1016/j.ijpharm.2016.11.040

Martinello, T., Kaneko, T. M., Velasco, M. V. R., Taqueda, M. E. S., & Consiglieri, V. O. (2006). Optimization of Poorly Compactable Drug Tablets Manufactured By Direct Compression Using The Mixture Experimental Design. *International Journal of Pharmaceutics*, *322*(1–2), 87–95. https://doi.org/10.1016/j.ijpharm.2006.05.034

USP. (2016). *The United States Pharmacopeia 39-The National Formulary 34*.

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Cost-utility analysis for non-small cell lung cancer with epidermal growth factor receptor mutations

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Abstract

Introduction: Lung cancer remains a leading cause of death worldwide, with non-small cell lung cancer (NSCLC) carrying the highest incidence among lung cancer types, particularly in patients with epidermal growth factor receptor (EGFR) mutations. The first-line therapy for NSCLC lung cancer patients with EGFR mutations typically involves the tyrosine kinase inhibitor (TKI) group.

Objective: The study aimed to compare the cost utility of the two TKI groups (afatinib and erlotinib) for NSCLC patients with EGFR mutations.

Method: This pharmacoeconomic study was conducted using the Cost Utility Analysis (CUA) method from the patient's perspective. The study included 33 patients receiving afatinib and 12 patients receiving erlotinib, conducted in 2022-2023 at Kariadi Hospital, Semarang, and Prof. Dr. Margono Soekarjo Hospital, Purwokerto. Therapeutic outcomes were measured using Quality Adjusted Life Year (QALY), and cost components included actual costs and estimated costs based on clinical pathways. CUA was presented in the form of an Incremental Cost Effectiveness Ratio (ICER).

Results: The cost of afatinib was greater than that of erlotinib (IDR 174,395,847 vs. IDR 138,672,688). The quality of life of afatinib was better than that of erlotinib (0.397 vs. 0.161, *p*-value 0.016). The ICER of afatinib was IDR 151,369,318 per QALY when compared to erlotinib.

Conclusion: This study demonstrates that afatinib is a cost-effective treatment option for NSCLC patients with EGFR mutations.

Keywords: Afatinib, EGFR, erlotinib, ICER, mutation

1. Introduction

The increasing number of deaths from lung cancer is a growing concern globally. According to the World Health Organization (WHO), lung cancer was responsible for 2,206,671 deaths in 2020, accounting for 11.4% of all cancer-related fatalities (Lukeman, 1976; Globocan, 2020). In the United States, lung cancer deaths caused 1,796,144 deaths, or 18% of the total number of cancer cases, while in Asia, deaths caused by lung cancer reached 1,315,136 cases, or 19.2% of the total number of cancer cases (Globocan, 2020; Globocan Asia, 2021). Meanwhile, in Indonesia, lung cancer was the leading cause of cancer-related deaths, with 11.4% of the country's cancer cases in 2020 and a male-to-female ratio of 13:1 (Joseph & Rotty, 2020; Sutnick & Gunawan, 1982).

The incidence of lung cancer cases with the type Non-Small Cell Lung Cancer (NSCLC) with Epidermal Growth Factor Receptor (EGFR) mutations varies (Cagle *et al*., 2013). The proportion of NSCLC cases in Indonesia is considered relatively high, with a proportion of more than 70% of the total lung cancer cases (Robot *et al.*, 2021). This type of cancer often leads to chronic symptoms such as prolonged coughing, with eventual mortality due to lung failure. In addition to the impact of physical conditions, other impacts include more complex medical therapy than other types.

Effective therapy for lung cancer patients with EGFR mutations can improve survival rates and reduce disease severity. First-line therapy for NSCLC lung cancer patients with EGFR mutations is the tyrosine kinase inhibitor (TKI) group, such as erlotinib, gefitinib, and afatinib (de Man *et al*., 2019; Novello *et al*., 2016). These three drugs are used to treat EGFR-positive mutations, including exon 19 and exon 21 L858R deletions (Maemondo *et al*., 2010). Among them, afatinib and erlotinib are the most commonly used tyrosine kinase inhibitors from the TKI group because they are more effective than other chemotherapy drugs (Nasrulsyah *et al*., 2020; Yang *et al*., 2017). Afatinib is also considered superior because it blocks the formation of protein kinases through ligands in cancer cells, preventing the early stages of cancer cell formation (Kim *et al*., 2021). Clinical data showed that for 467 NSCLC patients with positive EGFR mutations, afatinib provided a longer Progression-Free Survival (PFS) (19.1 months) compared to erlotinib (7.1 months) and gefitinib (14 months) (Brueckl *et al*., 2018; Kim *et al*., 2021; F. Wulandari *et al*., 2021; Yang *et al*., 2017).

The presence of mutations in lung cancer patients not only complicates treatment but also increases costs. The effectiveness and efficiency of the treatment are a growing concern for the government, especially in the implementation of the National Health Insurance (JKN) program (Hadiningsih, 2015). Previous research indicates that the average cost for a lung cancer patient undergoing first-line chemotherapy is IDR 14,561,930 (Wulandari *et al*., 2019). Moreover, the cost of lung cancer is considered relatively expensive compared to other cancers when viewed from the comparison of the cost/Progression-Free Survival (PFS) ratio. The cost of erlotinib is IDR 1,693,139/month, gefitinib is IDR 1,227,323/month, and afatinib is IDR 2,429,366/month. Of these, gefitinib therapy is the most economical TKI, followed by erlotinib and afatinib. However, when compared to standard therapy, the average cost of the TKI group is more expensive (IDR 12,000,000) than the cost of standard therapy (IDR 8,869,645) (Nurhayati *et al*., 2021; Wulandari *et al*., 2019).

Given these concerns, this study aims to compare the two TKI treatments (afatinib with erlotinib) to assess the cost-utility of these therapies. This comparison is crucial to guide Health Technology Assessment (HTA) activities in Indonesia in lung cancer patients with EGFR mutations.

2. Methods

2.1. Description of materials and sample collection techniques

This pharmacoeconomic study was conducted using the Cost Utility Analysis (CUA) from the patient perspective, focusing on NSCLC lung cancer patients with EGFR mutations. This study was conducted on patients receiving TKI therapy between 2022 and 2023 at Kariadi General Hospital Semarang and Prof. Dr. Margono Soekarjo General Hospital Purwokerto. Ethical approval was

granted by the Health Research Ethics Commission of Universitas Muhammadiyah Purwokerto with the ethical eligibility number KEPK/UMP/101/I/2023.

Purposive sampling was carried out to select patients who met the following inclusion criteria: patients diagnosed with NSCLC lung cancer had EGFR mutations, received TKI therapy, and had received at least three therapy sessions. The exclusion criteria in this study were patients with incomplete medical record data and patients who did not complete treatment during the study period. The number of samples taken according to the inclusion-exclusion criteria was 45 patients: 33 patients receiving afatinib therapy and 12 patients receiving erlotinib therapy.

2.2. Research process description

Patient characteristics, such as gender, age, education, occupation, and health insurance, were collected from patient medical records. In comparison, quality of life data was obtained from interviews with patients using EQ-5D-5L (EuroQol 5 Dimensions 5 Levels), which has been standardized in Indonesia. The five dimensions measured in the survey were mobility, self-care, daily activities, pain/discomfort, and anxiety/depression, with the level of statements reported by the patients themselves. The responses are classified into five levels: no difficulty, a little difficulty, quite a bit of difficulty, very difficult, and unable. The results of quality-of-life data were utilities calculated in the Indonesian value set (Hamida *et al*., 2019).

The cost data collected in this study included direct medical, direct non-medical, and indirect cost data. Direct medical costs were obtained from patient billing at the hospital, covering drugs and medical devices, laboratories, accommodation, and doctor visits. The cost data were then grouped into costs when patients underwent surgery, targeted therapy, and radiotherapy (Restyana & Admaja, 2019). Direct non-medical cost data included parking, food, and transportation costs collected through interviews. Indirect costs, including productivity loss, were also collected through interviews. The three types of cost data were classified as actual costs, where this data describes the real costs incurred during therapy. Furthermore, the actual cost data obtained from the hospital and the patient were weighted by the clinical pathway obtained from The National Comprehensive Cancer Network (NCCN) to calculate the unit cost required by patients when diagnosed with lung cancer. Based on the 2020 NCCN guidelines, patients are recommended to undergo surgery once, target therapy six times, and radiotherapy 30 times (Ettinger *et al*., 2021).

2.3. Statistical analysis

The statistical analyses used in this study were chi-square, independent sample t-test, and cost-effectiveness. Chi-square analysis was used to test the difference between the characteristics of the afatinib and erlotinib groups. Independent sample t-test analysis was utilized to test the difference in numerical data on the age and utility variables for the use of afatinib and gefitinib therapies. Cost-effectiveness analysis was calculated using the Incremental Cost Effectiveness Ratio (ICER) (Ardhila *et al*., 2024), whose value was compared to the Willingness to Pay (WTP) in Indonesia. The determination of the WTP value is based on 3 times the Gross Domestic Product (GDP) per capita in 2021, which is 186.6 million/utility index (Atikasari *et al*., 2023).

3. Result and discussion

3.1. Characteristics patient

The demographic characteristics of NSCLC lung cancer patients with EGFR mutations in afatinib and erlotinib groups show similarities in terms of gender, age, education level, occupation, and health insurance categories used (p-value > 0.05) (**Table 1**). Most patients were female (24 people (53.33%)), which corroborates findings by Fujiwara *et al*. (2018), who report that NSCLC is more prevalent among women. The estrogen hormone might influence lung cell differentiation and EGFR activation, contributing to this higher incidence in women (Fujiwara *et al*., 2018; Hsu *et al*., 2017).

The average age of lung cancer patients with EGFR mutations was 52 years, classifying them as older adults. It is in line with previous studies suggesting that older adults are vulnerable to being the main factor (Fujiwara *et al*., 2018; Oktaviyanti, 2015). Another theory also states that age affects the occurrence of oncogenesis, so increasing age affects genetic changes that have an impact on the decline in stem cell function. In normal individuals, stem cells can act as a barrier to tumor growth by creating an environment that is not conducive to gene mutations. Age-related declines in stem cell function may increase the risk of genetic mutations, including EGFR mutations, due to reduced tumor-suppressive barriers (Tezel *et al*., 2017).

In this study, the highest level of patient education was at the high school level, the majority of patient education was in high school (23 patients (250.11%)). Based on research findings by Sulviana and Kurniasari (2021), 140 out of 216 cancer patients are in high school. While higher education levels are not directly linked to cancer risk, unhealthy lifestyles can elevate the risk regardless of education level (Balatif & Sukma, 2021). Meanwhile, the majority of lung cancer patients with EGFR mutations are private employees, most were private employees 15 (33.3%).

According to research by Pritami *et al*. (2022), the type of work can increase the risk of lung cancer. Exposure to air pollution and chemicals from the work environment can trigger lung cancer. However, these factors are not found to significantly contribute to EGFR mutation-related NSCLC (Putriani *et al*., 2019). Meanwhile, health insurance does not affect therapy coverage; it affects the patient's source of funds.

Table 1. Respondent characteristics

Variable	Afatinib (N=33)		Erlotinib (N=12)		Total (N=45)		*P*-value
	N	%	N	%	N	%	
Gender							
Female	16	48.5	8	66.7	24	53.33	0.679
Male	17	51.5	4	33.3	21	46.67	
Age							
Mean (SD)	52.52 (11.97)		53.3 (12.37)		52.73 (11,94)		0.842
Median (min: max)	52 (21:71)		55 (21:70)		54 (21:71)		
Education							
Bachelor	6	18.2	4	33.3	10	22.22	0.691
Senior High School	20	60.6	3	25	23	50.11	
Junior High School	4	12.1	3	25	7	15.56	
Elementary School	3	9.1	2	16.7	5	11.11	
Occupation							
Work	21	63.6	11	97.67	32	71.1	0.377
Not working	12	36.4	1	8.3	13	28.9	
Health assurance							-
BPJS	31	93.90	12	100	43	95.56	
Non-BPJS	2	6.1	-		2	4.44	

3.2 Quality of live of patients

The quality of life of patients in both therapy groups illustrated different utility values (**Table 2**). The quality of life of the afatinib group was higher than that of gefitinib (0.397 vs. 0.161, *p*-value 0.016). However, the VAS value was only found in the afatinib group (77.667 ± 4.169). Dwilovianita *et al*. (2022) reported that 85% of cancer patients experienced pain or discomfort, while 36.7% had mild anxiety, 23.3% had moderate anxiety, and 46% had severe anxiety. This pain is attributed to the effects of the drug or from the cancer itself. Increased anxiety occurs due to patients' fear of their future as they face lung cancer. Patients feel anxious because of medical procedures, such as surgery, chemotherapy, and target therapy or hormone therapy, especially when these treatments are prolonged (Dwilovianita *et al*., 2022).

Tabel 2. Quality-of-life

Dimensions	Levels	Afatinib (n=23)	Erlotinib (n=4)	Total (n=27)
Mobility	No problem	4 (17.4%)	0 (0%)	4 (14.8%)
	There is a problem	19 (82.6%)	4 (100%)	23 (85.2%)
Self-care	No problem	7 (30.4%)	0 (0%)	7 (25.9%
	There is a problem	16 (69.6%)	4 (100%)	20 (74.1%)

Dimensions	Levels	Afatinib (n=23)	Erlotinib (n=4)	Total (n=27)
Daily activity	No problem	1 (4.3%)	0 (0%)	1 (3.7%)
	There is a problem	22 (95.7)	4 (100%)	26 (96.3)
Pain/discomfort	No problem	0 (0%)	0 (0%)	0 (0%)
	There is a problem	23 (100%)	4 (100%)	27 (100%)
Anxiety/depression	No problem	0 (0%)	0 (0%)	0 (0%)
	There is a problem	23 (100%)	4 (100%)	27 (100%)
Utility	Mean ± SD	0.397 ± 0.172	0.161 ± 0.145	*p-Value* 0.016
VAS	Mean ± SD	77.667 ± 4.169	-	-

3.3 Cost analysis

The actual total cost required for NSCLC lung cancer patients with EGFR mutations to maintain treatment using afatinib was higher than for erlotinib patients (IDR 36,460,407 ± 18,686,717 vs. IDR 34,052,431 ± 15,944,215). It was primarily due to the direct medical costs of NSCLC lung cancer patients with EGFR mutations using afatinib were higher than those of erlotinib (IDR 35,819,553 ± 18,028,849 vs. IDR 33,560,764 ± 15,440,757). However, the direct non-medical costs of afatinib therapy were lower than those of erlotinib (IDR 137,521 ± 152,436 vs. IDR 217,500 ± 130,032). Additionally, the indirect costs of therapy with afatinib were higher than those of erlotinib (IDR 503,333 ± 505,432 vs. IDR 274,167 ± 373,426) (**Table 3**). Patients receiving gefitinib 150 required lower costs than the afatinib group (IDR 36,460,407 ± IDR 18,686,717 vs. IDR 34,052,431 ± IDR 15,944,215). It aligns with previous studies indicating that additional costs for afatinib therapy are higher ($9745) than those of gefitinib and erlotinib (Kim *et al*., 2021). Other factors determining the difference in total costs are the frequency of visits to the hospital and the existence of supporting examinations, which contribute to administrative and service costs (Nurhayati *et al*., 2021).

3.3. Incremental Cost Effectiveness Ratio (ICER)

A cost analysis based on clinical pathways discovered that the total cost of afatinib therapy was higher than that of erlotinib (IDR 174,395,847 vs. IDR 138,672,688) (**Table 4**). The Incremental Cost-Effectiveness Ratio (ICER) for afatinib administration was calculated to be IDR 151,369,318/ Quality-Adjusted Life Year (QALY) when compared with erlotinib. This study was consistent with the study by You *et al*. (2021), who reported that the direct medical costs of afatinib were significantly higher ($35,808) compared to erlotinib ($18,724). Similarly, Gu *et al*. (2019) found that afatinib is more expensive ($43,629), than erlotinib ($40,811).

Table 3. Cost analysis

Cost Component	Afatinib		Erlotinib		All Patients	
	Mean (IDR)	SD (IDR)	Mean (IDR)	SD (IDR)	Mean (IDR)	SD (IDR)
Surgery		**N=6**		**N=5**		**N=11**
Pharmacy	2,624,344	1,112,090	3,567,624	2,321,376	3,095,984	1,716,733
Laboratories	2,640,017	1,452,727	2,562,360	1,955,647	2,601,189	1,704,187
Accommodation	5,200,000	3,108,054	6,130,000	4,969,859	5,665,000	4,038,957
Visited the doctor	3,040,000	753,870	2,388,000	2,006,370	2,714,000	1,380,120
Administration	31,000	5,292	35,600	7,603	33,300	6,448
Medical treatment	3,831,083	2,545,174	5,799,100	970,458	4,815,092	1,757,816
Care treatment	842,000	199,721	694,600	578,167	768,300	388,944
Radiology	2,725,583	1,798,443	2,161,700	1,382,518	2,443,642	1,590,481
Total cost	20,934,027	10,975,371	23,338,984	14,191,998	22,136,506	12,583,685
Targeted therapy		**N=33**		**N=12**		**N=42**
Registration	15,152	14,169	26,250	2,261	20,701	8,215
Pharmacy	1,771,302	2,665,498	222,947	90,937	997,125	1,378,218
Laboratories	119,367	50,965	91,333	4,677	105,350	27,821
Targeted therapy	10,545,891	2,720,162	8,113,333	9,847	9,329,612	1,365,005
Radiology	1,149,470	786,405	501,125	555,242	825,298	670,824
Total cost	13,601,182	6,237,199	8,954,988	662,964	11,278,085	3,450,082
Radiotherapy		**N=18**		**N=12**		**N=30**
Registration	17,273	11,531	26,250	2,261	21,762	6,896
Laboratories	94,985	5,803	89,750	4,961	92,367	5,382
Radiotherapy	736,364	93,602	825,000	45,227	780,682	69,415
Radiology (CT-Scan)	435,722	705,343	325,792	533,346	380,757	619,345
Total cost	1,284,344	816,279	1,266,792	585,795	1,275,568	701,037
Total direct medical cost	**35,819,553**	**18,028,849**	**33,560,764**	**15,440,757**	**34,690,158**	**16,734,803**
Direct non-medical cost		**N=23**		**N=4**		**N=27**
Parking	4,913	5,239	5.000	0	4,957	2,620
Food	38,478	13,180	67,500	22,174	52,989	17,677
Transportation	94,130	134,017	145,000	107,858	119,565	120,938
Total direct non-medical cost	**137,521**	**152,436**	**217,500**	**130,032**	**177,511**	**141,234**
Indirect cost		**N=23**		**N=4**		**N=27**
Productivity loss	503,333	505,432	274,167	373,426	388,750	439,429
Total indirect cost	**503,333**	**505,432**	**274,167**	**373,426**	**388,750**	**439,429**
TOTAL COST	**36,460,407**	**18,686,717**	**34,052,431**	**15,944,215**	**35,256,149**	**17,315,466**

This study demonstrated that the use of afatinib for NSCLC lung cancer patients with EGFR mutations was a cost-effective strategy when compared to erlotinib. Although the cost of afatinib was higher than erlotinib, patients receiving afatinib reported a better quality of life than those treated with erlotinib. The determination of the WTP value is based on 3 times the Gross Domestic Product (GDP) per capita in 2021, which is 186.6 million/utility index (Atikasari *et al*., 2023). This finding aligns with previous research indicating that afatinib therapy provides higher effectiveness and QALY values than erlotinib (Kim *et al*., 2021; Zhu *et al.*, 2018). Moreover, afatinib is considered a cost-

saving strategy compared with erlotinib and gefitinib for EGFR mutation-positive NSCLC patients (Kim *et al.*, 2021; Zhu *et al*., 2018).

Tabel 4. Cost analysis based on clinical pathway

Treatment	Afatinib				Erlotinib				ICER
	Cost (IDR)	Quantity	Total (IDR)	QALYs	Cost (IDR)	Quantity	Total (IDR)	QALYs	
Surgery	20,934,027	1	20,934,027		23,338,984	1	23,338,984		
Targeted therapy	13,601,182	6	81,607,092		8,954,988	6	53,729,928		
Radiotherapy	1,284,344	30	38,530,320		1,266,792	30	38,003,760		
Direct-nonmedical cost	137,521	52	7,151,092		217,500	48	10,440,000		
Non-direct cost	503,333	52	26,173,316		274,167	48	13,160,016		
Total			**174,395,847**	**0.397**			**138,672,688**	**0.161**	**151,369,318**

One of the limitations of this study was the lack of data on the toxicity profiles and side effects of each treatment, which could affect the results of the patient's quality of life. Further research is needed, including the assessment of Drug Related Problems (DRP), as cancer patients often experience more side effects. Data on quality-of-life subjects were limited due to the challenges in directly interviewing patients. Future studies could involve other patients in similar conditions to increase the number of respondents. VAS scores were missing for one group due to differences in enumerator perceptions during data collection; this issue could be mitigated by standardizing data collection. Another limitation was the small sample size; therefore, further research should include a larger and more diverse population across multiple research locations in Indonesia. Comparative studies on other TKI groups, such as gefitinib, could also provide a more comprehensive cost-utility profile of TKI group therapies.

5. Conclusion

The administration of afatinib is considered to be more cost-effective than erlotinib for NSCLC lung cancer patients with EGFR mutations.

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References

Ardhila, N. F., Endarti, D., & Phodha, T. (2024). Systematic Review of Economic Evaluation Studies of Stroke Disease: Cost Effectiveness. *Journal of Health Economic and Policy Research (JHEPR)*, *2*(1), 27–34. https://doi.org/10.30595/jhepr.v2i1.110

Atikasari, V., Setiawan, D., Prasuma, G. S., & Sugiantoro, E. A. (2023). Analisis Efektivitas Biaya Jamu Saintifik Pada Pasien Osteoarthritis di Indonesia. *JPSCR: Journal of Pharmaceutical Science and Clinical Research*, *8*(1), 129–138. https://doi.org/10.20961/jpscr.v8i1.59797

Balatif, R., & Sukma, A. A. M. (2021). Memahami Kaitan Gaya Hidup dengan Kanker: Sebagai Langkah Awal Pencegahan Kanker. *SCRIPTA SCORE Scientific Medical Journal*, *3*(1), 40–50. https://doi.org/10.32734/scripta.v3i1.4506

Brueckl, W. M., Achenbach, H. J., Ficker, J. H., & Schuette, W. (2018). Erlotinib Treatment After Platinum-Based Therapy In Elderly Patients With Non-Small-Cell Lung Cancer In Routine Clinical Practice - Results From The Eldertac Study. *BMC Cancer*, *18*(333), 1–11. https://doi.org/10.1186/s12885-018-4208-x

Cagle, P. T., Allen, T. C., & Olsen, R. J. (2013). Lung Cancer Biomarkers Present Status and Future Developments. *Archives of Pathology & Laboratory Medicine*, *137*(9), 1191–1198. https://doi.org/10.5858/arpa.2013-0319-CR

de Man, Y., Atsma, F., Oosterveld-Vlug, M. G., Brom, L., Onwuteaka-Philipsen, B. D., Westert, G. P., & Groenewoud, A. S. (2019). The Intensity of Hospital Care Utilization by Dutch Patients With Lung or Colorectal Cancer in their Final Months of Life. *Cancer Control*, *26*(1), 1–9. https://doi.org/10.1177/1073274819846574

Dwilovianita, Y., Annisa, E., Parlaungan, B., Sihite, S., Pranata, H., & Ginting, C. N. (2022). Hubungan Karakteristik Nyeri Dan Kecemasan Pasien Yang Menjalani Kemoterapi. *Jurnal Penelitian Perawat Profesional*, *4*(1), 17–26.

Ettinger, D. S., Wood, D. E., Aisner, D. L., Akerley, W., Bauman, J. R., Bharat, A., Bruno, D. S., Chang, J. Y., Chirieac, L. R., D'Amico, T. A., Dilling, T. J., Dowell, J., Gettinger, S., Gubens, M. A., Hegde, A., Hennon, M., Lackner, R. P., Lanuti, M., Leal, T. A., … Hughes, M. (2021). Non-Small Cell Lung Cancer,Version 2.2021 Featured Updates to the NCCN Guidelines. *JNCCN Journal of the National Comprehensive Cancer Network*, *19*(3), 255–266. https://doi.org/10.6004/jnccn.2021.0013

Fujiwara, A., Yoshida, M., Fujimoto, H., Nakahara, H., Ito, K., Nishihama, K., Yasuma, T., Hataji, O., Taguchi, O., D'Alessandro-Gabazza, C. N., Gabazza, E. C., & Kobayashi, T. (2018). A Retrospective Comparison of the Clinical Efficacy of Gefitinib, Erlotinib, and Afatinib in Japanese Patients With Non-Small Cell Lung Cancer. *Oncology Research*, *26*(7), 1031–1036. https://doi.org/10.3727/096504018X15151523767752

Globocan, 2020. Lung Fact Sheet. Glob. Obs. Cancer 419, 1–2.

Globocan Asia, 2021. Asia : Globocan 2020 Summary Statistic. Int. Agency Res. Cancer 136, 2.

Gu, X., Zhang, Q., Chu, Y. B., Zhao, Y. Y., Zhang, Y. J., Kuo, D., Su, B., & Wu, B. (2019). Cost-effectiveness of Afatinib, Gefitinib, Erlotinib and Pemetrexed-Based Chemotherapy As First-Line Treatments For Advanced Non-Small Cell Lung Cancer In China. *Lung Cancer*, *127*(2019), 84–89. https://doi.org/10.1016/j.lungcan.2018.11.029

Hadiningsih, H. (2015). Analisis Besaran Biaya Obat Beberapa Penyakit Rawat Jalan dan Faktor-Faktor yang Mempengaruhi di Rs. Awal Bros Bekasi Tahun 2014. *Jurnal Administrasi Rumah Sakit Indonesia*, *2*(1), 53–63. https://doi.org/10.7454/arsi.v2i1.2188

Hamida, N., Ulfa, M., Haris, R. N. H., Endarti, D., & Wiedyaningsih, C. (2019). Pengukuran Kualitas Hidup Pasien Program Pengelolaan Penyakit Kronis (Prolanis) di Puskesmas Menggunakan Instrumen EQ-5D-5L. *Majalah Farmaseutik*, *15*(2), 67–74. https://doi.org/10.22146/farmaseutik.v15i2.46328

Hsu, L. H., Chu, N. M., & Kao, S. H. (2017). Estrogen, Estrogen Receptor and Lung Cancer. *International Journal of Molecular Sciences*, *18*(1713), 1–17. https://doi.org/10.3390/ijms18081713

Joseph, J., & Rotty, L. W. A. (2020). Kanker Paru: Laporan Kasus. *Medical Scope Journal (MSJ)*, *2*(1), 17–25. https://doi.org/10.1155/2019/9303170

Kim, Y. J., Oremus, M., Chen, H. H., McFarlane, T., Fearon, D., & Horton, S. (2021). Cost-Effectiveness Analysis of Afatinib, Erlotinib, and Gefitinib as First-Line Treatments for EGFR Mutation-Positive Non-Small-Cell Lung Cancer in Ontario, Canada. *PharmacoEconomics*, *39*(5), 537–548. https://doi.org/10.1007/s40273-021-01022-9

Lukeman, J. M. (1976). What Is Lung Cancer? *Perspectives in Lung Cancer*, 30–40. https://doi.org/10.1159/000400400

Maemondo, M., Inoue, A., Kobayashi, K., Sugawara, S., Oizumi, S., Isobe, H., Gemma, A., Harada, M., Yoshizawa, H., Kinoshita, I., Fujita, Y., Okinaga, S., Hirano, H., Yoshimori, K., Harada, T., Ogura, T., Ando, M., Miyazawa, H., Tanaka, T., … Nukiwa, T. (2010). Gefitinib or Chemotherapy for Non–Small-Cell Lung Cancer with Mutated EGFR. *New England Journal of Medicine*, *362*(25), 2380–2388. https://doi.org/10.1056/nejmoa0909530

Nasrulsyah, C., Asyura, F., Hasan, K., Maulidani, & Sofia, M. (2020). Analisis SWOT Dalam Mengatasi Kelemahan Dan Kekurangan Rumah Sakit Siloam. *Makma*, *3*(3), 295–305.

Novello, S., Barlesi, F., Califano, R., Cufer, T., Ekman, S., Levra, M. G., Kerr, K., Popat, S., Reck, M., Senan, S., Simo, G. V., Vansteenkiste, J., Peters, S., & on behalf of the ESMO Guidelines Committee. (2016). Metastatic Non-Small-Cell Lung Cancer: ESMO Clinical Practice Guidelines For Diagnosis, Treatment and Follow-Up. *Annals of Oncology*, *27*(Supplement 5), 1–27. https://doi.org/10.1093/annonc/mdw326

Nurhayati, F., Anggriani, Y., Syahruddin, E., Ramadaniati, H. U., & Kusumaeni, T. (2021). Cost-effectiveness Analysis of Tyrosine Kinase Inhibitors (Erlotinib vs. Gefitinib vs. Afatinib) In Non-Small-Cell Lung Cancer. *Journal of Applied Pharmaceutical Science*, *11*(4), 88–95. https://doi.org/10.7324/JAPS.2021.110411

Oktaviyanti, I. K. (2015). Mutasi EGFR Pada Pemeriksaan Sitologi Adenokarsinoma Paru. *Berkala Kedokteran*, *11*(2), 213–219. https://ppjp.ulm.ac.id/journal/index.php/jbk/article/view/171

Pritami, A. A., Soemarwoto, R. A. S., & Wintoko, R. (2022). Faktor Risiko Kanker Paru : Tinjauan Pustaka. *Agromedicine*, *9*(2), 120–123.

Putriani, F. A., Kholis, F. N., & Purwoko, Y. (2019). Perbedaan Faktor Risiko Penderita Adenokarsinoma Paru Dengan Mutasi EGFR Dan Non Mutasi EGFR. *Jurnal Kedokteran Diponegoro*, *8*(1), 214–221.

Restyana, A., & Admaja, W. (2019). Analisa Biaya Penggunaan Seftriakson dan Siprofloksasin Pasien Infeksi Saluran Kemih di Rumah Sakit X Kabupaten Jombang Tahun 2017. *PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia)*, *16*(2), 347–355. https://doi.org/10.30595/pharmacy.v16i2.5847

Robot, R. Y., Durry, M. F., & Kairupan, C. F. (2021). Morfologi, Patogenesis, dan Imunoterapi Kanker Paru Tipe Adenokarsinoma. *Medical Scope Journal*, *3*(1), 74–82. https://doi.org/10.35790/msj.3.1.2021.33544

Sulviana, E. R., & Kurniasari, L. (2021). Hubungan Antara Usia, Pendidikan, dan Pekerjaan dengan Kejadian Kanker Payudara pada Wanita di Kalimantan Timur. *Borneo Student Research*, *2*(3), 1937–1943. https://journals.umkt.ac.id/index.php/bsr/article/download/1988/951

Sutnick, A. I., & Gunawan, S. (1982). Cancer in Indonesia. *Journal of the American Medical Association*, *247*(22), 3087–3088. https://doi.org/10.1001/jama.247.22.3088

Tezel, G. G., Şener, E., Aydın, Ç., & Önder, S. (2017). Prevalence of Epidermal Growth Factor Receptor Mutations in Patients with Non-Small Cell Lung Cancer in Turkish Population. *Balkan Medical Journal*, *34*(6), 567–571. https://doi.org/10.4274/balkanmedj.2017.0297

Wulandari, A., Monalisa, S., & Zaini, J. (2019). Analisis Biaya Kemoterapi Lini Pertama Pada Pasien Kanker Paru di Rumah Sakit Persahabatan Jakarta Timur Periode Tahun 2016. *Sainstech Farma Jurnal Ilmu Kefarmasian*, *12*(2), 85–92.

Wulandari, F., Utami, W., Rohana, E., & Prabhata, W. R. (2021). Efikasi Terapi Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitor (EGFR-TKIs) pada Kanker Paru. *Journal of Research in Pharmacy*, *1*(1), 24–31. https://doi.org/10.2493/jjspe.87.947

Yang, Z., Hackshaw, A., Feng, Q., Fu, X., Zhang, Y., Mao, C., & Tang, J. (2017). Comparison of Gefitinib, Erlotinib and Afatinib In Non-Small Cell Lung Cancer: A Meta-Analysis. *International Journal of Cancer*, *140*(12), 2805–2819. https://doi.org/10.1002/ijc.30691

You, J. H. S., Cho, W. C. S., Ming, W., Li, Y., Kwan, C., Au, K., & Au, J. S. (2021). EGFR Mutation-Guided Use of Afatinib, Erlotinib and Gefitinib For Advanced Non-Small-Cell Lung Cancer In Hong Kong - A Cost-Effectiveness Analysis. *PLoS ONE*, *16*(3), 1–14. https://doi.org/10.1371/journal.pone.0247860

Zhu, J., He, W., Ye, M., Fu, J., Chu, Y. B., Zhao, Y. Y., Zhang, Y. J., Kuo, D., & Wu, B. (2018). Cost-effectiveness of Afatinib and Erlotinib As Second-Line Treatments For Advanced Squamous Cell Carcinoma of The Lung. *Future Oncology*, *14*(27), 2833–2840. https://doi.org/10.2217/fon-2018-0321

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Activity of Superoxide Dismutase (SOD) in guava (*Psidium guajava L.*) leaf extracts using the Water Soluble Tetrazolium Salt-1 (WST-1) method

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Abstract

Background: Antioxidants are compounds that inhibit cell damage caused by free radicals. Antioxidant enzymes include superoxide dismutase (SOD), glutathione, and catalase. SOD protects cells against free radicals implicated in various diseases. The guava plant (*Psidium guajava* L.) is known to possess SOD activity.

Objective: This research was performed to determine the activity of the superoxide dismutase enzyme in guava leaves extract (*Psidium guajava L.*) with ammonium sulfate concentrations of 25, 50, and 75%.

Methods: Guava leaves were extracted using phosphate buffer and centrifugation. The superoxide dismutase (SOD) enzyme was then purified using ammonium sulfate at concentrations of 25, 50, and 75%. The amount of protein was measured with the Lowry method, and the activity of the SOD enzyme was tested using the WST-1 assay to find out how much it was inhibited. The resulting data were statistically analyzed using one-way ANOVA.

Results: Guava leaves extract exhibits SOD enzyme activity. The total protein content of the crude guava leaf extract and the extracts purified with 25, 50, and 75% ammonium sulfate were 9.683, 6.958, 8.842, and 11.269 mg/mL, respectively. The corresponding percent inhibition values were 69.652, 35.323, 61.69, and 79.104%. The 75% ammonium sulfate concentration yielded the highest percent inhibition.

Conclusion: Guava leaves extract exhibits SOD enzyme activity, with the highest percent inhibition observed at an ammonium sulfate concentration of 75%.

Keywords: *Psidium guajava* L., free radicals, antioxidants, SOD, WST-1

1. Introduction

Contemporary lifestyles including unhealthy dietary patterns have adverse health implications. Furthermore, environmental pollution can reduce the quality of life due to lower production of compounds essential for physiological function. Natural antioxidants help maintain the health by mitigating the detrimental effects of free radicals generated by various environmental stressors, such as air pollution, radiation, hazardous chemicals, cigarette smoke, and solar radiation (Arnanda & Nuwarda, 2019).

Free radicals with unpaired electrons have high reactivity as they grab electrons from the surrounding molecules. This process induces oxidative stress, causing an imbalance between free radical production and antioxidant defenses and cellular damage. Oxidative stress occurs when the generation of reactive oxygen species (ROS) exceeds the available antioxidant capacity and overwhelm the cellular detoxification systems (Arief & Widodo, 2018).

Antioxidant counteracts the effects from free radicals by suppressing molecular oxidation. It neutralizes and inhibits the oxidation reactions that involve free radicals (Bintarti, 2014). Endogenous antioxidants, such as superoxide dismutase (SOD), offer a vital defense against oxidative

stress (Werdhawati, 2014) and protects cells from free radical-mediated damage, mitigating the risk of various diseases (Yanti *et al*., 2016). Furthermore, SOD may enhance the therapeutic efficacy of certain pharmaceuticals and holds potential as a nutritional supplement (Younus, 2018).

SOD can be obtained from plants that contain antioxidant compounds. Guava (*Psidium guajava L)* inhibits free radicals (Maulana *et al*., 2016) from its antioxidant compounds and it exhibits high SOD that reaches 100% in its leaves (Widowati *et al*., 2005). This research was performed to investigate the SOD activity, total protein content, and percentage inhibition of crude guava leaves extract purified with 25, 50, and 75% ammonium sulfate concentrations to determine the optimal purification concentration for SOD inhibition.

2. Method

2.1. Tools and materials

This research utilized the following tools: blender, analytical balance, knife, pH meter, measuring cup, beakers, Erlenmeyer flasks, refrigerator, centrifuge, flannel, jars, micropipettes, volumetric pipettes, volumetric flasks, magnetic stirrer, UV-Vis spectrophotometer, 96-well plate, incubator, and microplate reader. The materials included guava leaves, phosphate buffer, ammonium sulfate, distilled water (aquades and aquabides), bovine serum albumin (BSA), WST solution, vitamin C, Na_2CO_3, NaOH, sodium potassium tartrate, $CuSO_4 \cdot 5H_2O$, Folin-Ciocalteu phenol reagent, assay buffer, enzyme solution, and vitamin C.

2.2. Sampling and identification

Plant identification was conducted at the Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT) in Tawangmangu, Karanganyar Regency, Central Java, to ensure accurate species identification and prevent sample contamination (Klau & Hesturini, 2021). Guava (*Psidium guajava L.*) leaves were collected from a single, healthy tree in Plagan village, Klaten Regency, Central Java. Only undamaged, fresh leaves were selected.

2.3. SOD enzyme extraction

One hundred grams of guava leaves were cut into small pieces and homogenized in a 1:2 ratio with 0.02M phosphate buffer (pH 7.2) using a blender. The homogenate was filtered through flannel, cooled overnight at <4°C, and then centrifuged at 5.000 rpm for 30 minutes at 4°C. The resulting supernatant was collected as the crude extract (Male *et al*., 2014).

2.4. Purification of SOD enzyme extract

Ammonium sulfate purification was performed at 25, 50, and 75% saturation. For each concentration, the appropriate amount of ammonium sulfate (4.02 g for 25%, 8.73 g for 50%, and 14.28 g for 75%) was gradually added to 30 mL of crude SOD extract while stirring with a magnetic stirrer at 4°C. Each mixture was then centrifuged at 5.000 rpm for 30 minutes at 4°C. Following centrifugation, the supernatant was discarded, and the resulting pellet was resuspended in phosphate buffer at a 1:2 ratio (Moon-Ai *et al*., 2012; Rahman *et al*., 2012).

2.5. The determination of total protein content using the Lowry method

Protein content was determined using the Lowry method (Lowry *et al*., 1951). Reagents were prepared as follows: reagent A (2% Na_2CO_3 in 0.10 N NaOH), reagent B (0.5% $CuSO_4 \cdot 5H_2O$ in 1% sodium potassium tartrate), reagent C (prepared fresh by mixing 50 mL of reagent A with 1 mL of reagent B), and reagent D (Folin-Ciocalteu phenol reagent diluted 1:1 with distilled water).

A BSA standard curve was generated using a 1000 ppm stock solution (50 mg BSA in 50 mL distilled water). Aliquots of the stock solution (0.5; 0.75; 1; 1.25; and 1.5 mL) were diluted to 10 mL with distilled water in volumetric flasks. One mL of each standard and a 100-fold diluted sample were treated as follows: 5 mL of reagent C was added, mixed, and incubated for 10 minutes. Then, 0.5 mL of reagent D was added, and the mixture was incubated for 25 minutes. Absorbance was measured at 750 nm using a UV-Vis spectrophotometer. Protein concentrations in the samples were determined using the linear regression equation derived from the BSA standard curve (Lowry *et al*., 1951).

2.6. SOD enzyme activity test using the Water Soluble Tetrazolium Salt-1 (WST-1) method

Crude extracts of guava leaves SOD, ammonium sulfate concentration, positive control, and negative control were added with 200 µL of WST Working Solution and 20 µL of enzyme working solution. **Table 1** shows the composition of sample solutions, blank 1, blank 2, and blank 3.

Table 1. Composition of sample solutions, positive and negative controls, and blanks (Qwele *et al*., 2013)

	Control (+) (µL)	**Control (-) (µL)**	**Sample (µL)**	**Blank 1 (µL)**	**Blank 2 (µL)**	**Blank 3 (µL)**
Sample solution			20		20	
ddH2O				20		20
WST working solution	200	200	200	200	200	200
Enzyme working solution	20	20	20	20		
Solution		20			20	20
Vitamin C	20					

The standard solutions, positive control, negative control, samples, blank 1, blank 2, and blank 3 were each added to a 96-well plate and incubated at 37°C for 20 minutes. Subsequently, the absorbance of each well was measured using a microplate reader at a wavelength of 450 nm (Qwele *et al.*, 2013). SOD activity was then calculated using the percentage inhibition in the following formula:

$$\%\ inhibition = \frac{(A\ blank\ 1 - A\ blank\ 3) - (A\ sample - A\ blank\ 2)}{(A\ blank\ 1 - A\ blank\ 2)}\ x100\%$$

Description :
A_{sample} =Sample absorbance
$A_{blank\ 1}$ = Blank 1 absorbance
A_{blank2} = Blank 2 absorbance
A_{blank3} = Blank 3 absorbance

2.7. Results analysis

Data was analyzed using a one-way ANOVA parametric statistical test. Prior to ANOVA, data normality was assessed using the Shapiro-Wilk test (significance level > 0.05), and homogeneity of variance was confirmed using Levene's test (significance level > 0.05). A Tukey post-hoc test was then performed to determine significant differences between groups.

3. Results and discussion

3.1. SOD enzyme extraction

Enzyme extraction aimed to separate the enzyme from guava leaf tissue cells, yielding a supernatant after centrifugation. Extracting SOD enzyme from 100 g of guava leaves with 200 mL of phosphate buffer resulted in 150 mL of supernatant and 20.541 g of sediment. The SOD enzyme was extracted by homogenizing the leaves with 0.02 M phosphate buffer (pH 7.2), using a buffer volume twice that of the leaf sample. This buffer addition maintains cell component integrity, preserving optimal cellular conditions (Arjita, 2009).

Whitaker (1994) mentioned that phosphate buffer maintains a neutral pH during intracellular enzyme extraction from plant tissue. Its properties do not interfere with protein binding or enzyme activity, making it suitable for this purpose. The homogenate was cooled overnight at 4°C before filtration to prevent enzyme degradation and allow residual leaf fibers to settle. Filtration using flannel separated the filtrate (containing cell components) from the residue (containing cell walls).

Subsequent centrifugation at 5000 rpm for 30 minutes separated the supernatant (crude enzyme extract) from remaining leaves fiber. Centrifugation separates components based on size and

molecular weight, and the low temperature minimizes enzyme activity loss (Masruroh *et al*., 2018). This process yielded two distinct fractions: the supernatant (containing the crude enzyme extract) and the sediment (impurities from the extraction process).

3.2. Purification using amonium sulfat

In this research, precipitation with ammonium sulfate was carried out at three concentrations: 25, 50, and 75%. The weights of the pellets resulting from partial purification were 0.1622 g at 25% ammonium sulfate, 0.7752 g at 50%, and 1.628 g at 75%.

These pellet weights were obtained from the precipitated protein during partial purification. The 75% ammonium sulfate concentration yielded the highest pellet weight, while the 25% concentration yielded the lowest. These partial purification pellet results showed that increasing the ammonium sulfate concentration resulted in more precipitated protein. Protein precipitation does not denature the protein; it only reduces its solubility. The protein can be concentrated by removing the remaining ammonium sulfate solution and redissolving the protein in an appropriate buffer solution (Wingfield, 2001).

By adding high concentrations of ammonium sulfate, water molecules attached to the protein's hydrophobic surface bind to the salt. As more water molecules bind to salt ions, the proteins interact, combine, and precipitate known as salting out (Bintang, 2010).

3.3. Protein measurement using Lowry method

Protein levels in this research were measured using the Lowry method. The maximum wavelength obtained was 750 nm, with an absorbance value of 0.5402, and the optimal operating time (OT) was determined to be 35–45 minutes. Bovine serum albumin (BSA) was used as a standard in the Lowry method, with a concentration series of 50, 75, 100, 125, and 150 ppm. BSA was chosen as a standard because it exhibits good linearity, produces an accurate standard curve, and is a widely used protein standard (Walker, 2002). **Table 3** presents the UV-Vis spectrophotometry absorbance values for the BSA concentration series.

Table 3. Absorbance values of BSA solution concentration series (*Bovine Serum Albumin*)

Concentration (ppm)	Absorbance
50	0.262
75	0.390
100	0.489
125	0.597
150	0.738

Standard solutions with several concentrations were prepared to determine the protein content in the samples. A linear regression equation was generated using the straight line obtained from the standard curve. This equation was based on the absorbance values (y) and the corresponding BSA concentrations (x) of the five BSA standard solutions.

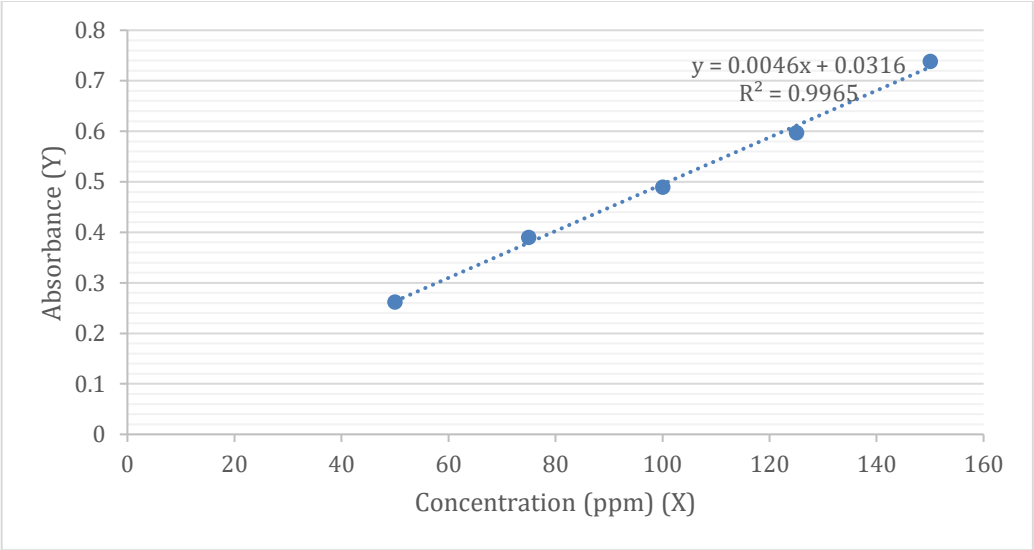

Figure 1. Linear regression equation of BSA absorbance concentration

The slope curve and the slope of the straight line are close to 1 (R^2 = 0.9965). The linear regression line equation y = a + bx and the equation y = 0.0316 + 0.0046x were obtained (**Figure 1**).

Table 4. Protein content results using the Lowry method

Sample	Content (mg/mL; mean ± SD)
Crude extract	9.683 ± 0.043
Ammonium sulfate 25%	6.958 ± 0.054
Ammonium sulfate 50%	8.842 ± 0.067
Ammonium sulfate 75%	11.269 ± 0.121

Based on the total protein content analysis, the pellets purified with 75% ammonium sulfate contained more protein than the crude extracts, as did the pellets purified with 25 and 50% ammonium sulfate. Higher concentrations of ammonium sulfate promote increased protein precipitation, which explains this result. SPSS analysis revealed a statistically significant difference in protein content among the crude extract and the samples purified with 25, 50, and 75% ammonium sulfate. This indicates that increasing the concentration of ammonium sulfate leads to a higher protein yield (Boschetti & Righetti, 2013).

The Lowry method is advantageous due to its 100-fold greater sensitivity compared to the biuret method, allowing for the use of smaller sample volumes. However, this increased sensitivity also makes the Lowry method more susceptible to interference, even though its detection limit is as low as 0.01 mg/mL (Purwanto, 2014).

3.4. SOD enzyme activity test

A quantitative activity assay was performed on the crude superoxide dismutase (SOD) extract from guava leaves to determine its SOD activity. A microplate reader was used for sample detection. This method was chosen due to its ability to accurately analyze small sample volumes (Qwele *et al*., 2013). The absorbance values for blanks 1, 2, and 3 were 0.168, 0.034, and 0.101, respectively.

The blank solutions served to calibrate the absorbance measurements. The variations in absorbance among the blanks likely reflect differences in their compositions. These blank absorbance values, along with the sample absorbance values, were then used to calculate the percentage inhibition using a specific formula. The resulting percentage inhibition values are presented in **Table 6**.

Table 6. Inhibition percentage of SOD enzyme

Sample	% of inhibition (Mean ± SD)
Positive Control	82.089 ± 1.492
Negative Control	2.985 ± 1.493
Crude extract	69.652 ± 3.756
Ammonium sulfate 25%	35.323 ± 2.279
Ammonium sulfate 50%	61.691 ± 2.280
Ammonium sulfate 75%	79.104 ± 1.492

Description:
Positive control : Vitamin C
Negative control : Buffer phosphate

The highest SOD activity was observed in the 75% ammonium sulfate purification, while the lowest activity was found in the 25% purification. The SOD activity at 75% ammonium sulfate was comparable to that of the positive control, indicating that the crude guava leaf extract retained substantial SOD activity at this concentration. This increase in activity is attributed to the higher purity of the enzyme obtained by increasing ammonium sulfate concentration.

The variation in SOD activity among samples reflects differences in superoxide anion reduction, which is linearly related to xanthine oxidase (XO) activity and inhibited by SOD. The SOD assay measures the reaction between WST-1 and superoxide radicals, producing yellow WST-1 formazan. SOD converts superoxide, thereby inhibiting the reduction of WST-1 to WST-1 formazan. SOD competes with WST-1 for superoxide, inhibiting dye formation. SOD activity is quantified by measuring the degree of inhibition of dye formation at 450 nm (Widowati *et al*., 2005). Vitamin C was used as a positive control due to its SOD-mimicking properties, while phosphate buffer served as a negative control, as it lacks SOD activity.

SPSS analysis confirmed SOD activity in the positive control, negative control, and all ammonium sulfate purifications (25, 50, and 75%). The negative control exhibited the lowest activity, while the 75% ammonium sulfate purification showed the highest, with no significant difference

observed between this purification and the positive control. Significant differences were found between the crude extract and each of the ammonium sulfate purifications (25, 50, and 75%).

4. Conclusion

Guava leaves extract (*Psidium guajava L.*) exhibits SOD activity. The total protein content in the crude SOD extract was 9.683 mg/mL. Following ammonium sulfate purification at 25, 50, and 75% concentrations, the protein content was 6.958, 8.842, and 11.269 mg/mL, respectively. The percentage inhibition values were 69.652% for the crude extract, and 35.323, 61.691, and 79.104% for the 25, 50, and 75% ammonium sulfate purifications, respectively. Therefore, the 75% ammonium sulfate concentration yielded the most optimal percentage inhibition.

References

Arief, H., & Widodo, M. A. (2018). Peranan Stres Oksidatif pada Proses Penyembuhan Luka. *Jurnal Ilmiah Kedokteran Wijaya Kusuma*, *5*(2), 22–29. https://doi.org/10.30742/jikw.v5i2.338

Arjita, I. P. D. (2009). Analisis Protein Jaringan Otak Sapi Dengan Metode Isolasi, Purifikasi dan Visualisasi. *GaneÇ Swara*, *3*(2), 55–58.

Arnanda, Q. P., & Nuwarda, R. F. (2019). Penggunaan Radiofarmaka Teknisium-99M Dari Senyawa Glutation dan Senyawa Flavonoid Sebagai Deteksi Dini Radikal Bebas Pemicu Kanker. *Farmaka Suplemen*, *17*(2), 236–243. https://jurnal.unpad.ac.id/farmaka/article/view/22071

Bintang, M. (2010). *Biokimia Teknik Penelitian*. Jakarta: Erlangga.

Bintarti, T. (2014). Skrining Fitokimia dan Uji Kemampuan Sebagai Antioksidan Dari Daun Jambu Biji (*Psidium guajava.* L). *Jurnal Ilmiah PANNMED (Pharmacist, Analyst, Nurse, Nutrition, Midwivery, Environment, Dentist)*, *9*(1), 40–44. https://doi.org/10.36911/pannmed.v9i1.341

Boschetti, E., & Righetti, P. G. (2013). *Low-Abundance Proteome Discovery*. Amsterdam: Elsevier Academic Press.

Klau, M. H. C., & Hesturini, R. J. (2021). Pengaruh Pemberian Ekstrak Etanol Daun Dandang Gendis (*Clinacanthus nutans* (Burm F) Lindau) Terhadap Daya Analgetik Dan Gambaran Makroskopis Lambung Mencit. *Jurnal Farmasi & Sains Indonesia*, *4*(1), 6–12. https://doi.org/10.52216/jfsi.v4i1.59

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein Measurement With The Folin Phenol Reagent. *The Journal of Biological Chemistry*, *193*(1), 265–275. https://doi.org/10.1016/s0021-9258(19)52451-6

Male, K. S., Nuryanti, S., & Rahmawati, S. (2014). Ekstrak Enzim Protease dari Daun Palado (*Agave angustifolia*) dan Pemanfaatannya dalam Prose Pembuatan Virgin Coconut Oil. *Jurnal Akademika Kimia*, *3*(3), 111–120.

Masruroh, H., Masruroh, U. D., Nugraheni, F. S., & Paramita, V. (2018). Analisa Kadar Lemak Dalam Susu Perah Sapi Menggunakan Gaya Sentrifugasi. *Metana*, *14*(1), 25–30. https://doi.org/10.14710/metana.v14i1.19172

Maulana, E. A., Astiti Asih, I. A. R., & Arsa, M. (2016). Isolasi dan Uji Aktivitas Antioksidan Senyawa Flavonoid Dari Ekstrak Daun Jambu Biji Putih (*Psidium guajava* Linn). *Jurnal Kimia*, *10*(1), 161–168. https://doi.org/10.24843/jchem.2016.v10.i01.p22

Moon-Ai, W., Niyomploy, P., Boonsombat, R., Sangvanich, P., & Karnchanatat, A. (2012). A Superoxide Dismutase Purified from the Rhizome of *Curcuma aeruginosa* Roxb. as Inhibitor of Nitric Oxide Production in the Macrophage-like RAW 264.7 Cell Line. *Applied Biochemistry and Biotechnology*, *166*(8), 2138–2155. https://doi.org/10.1007/s12010-012-9640-9

Purwanto, M. G. M. (2014). Perbandingan Analisa Kadar Protein Terlarut dengan Berbagai Metode Spektroskopi UV-Visible. In *Jurnal Ilmiah Sains & Teknologi*, *7*(2), 64–71.

Qwele, K., Hugo, A., Oyedemi, S. O., Moyo, B., Masika, P. J., & Muchenje, V. (2013). Chemical Composition, Fatty Acid Content and Antioxidant Potential of Meat From Goats Supplemented With Moringa (*Moringa oleifera*) Leaves, Sunflower Cake and Grass Hay. *Meat Science*, *93*(3), 455–462. https://doi.org/10.1016/j.meatsci.2012.11.009

Rahman, H., Kartawinata, G., & Julianti, E. (2012). Uji Aktivitas Enzim Superoksida Dismutase dalam Ekstrak Mesokarp Buah Merah (*Pandanus conoideus* Lamarck) Menggunanakan Densitometri Citra Elektroforegram. *Acta Pharmaceutica Indonesia*, *37*(2), 43–47.

Walker, J. M. (2002). *The Protein Protocols Handbook Second Edited*. United Kingdom: Humana Press Inc.

Werdhawati, A. (2014). Peran Antioksidan Bagi Kesehatan. *Biotek Medisiana Indonesia*, *3*(1), 59–68.

Whitaker, J. R. (1994). *Principle of Enzymology for The Food Science. Second Edition*. New York: Marcel Decker.

Widowati, W., Safitri, R., Rumumpuk, R., & Siahaan, M. (2005). Penapisan Aktivitas Superoksida Dismutase pada Berbagai Tanaman. *Jurnal Kedokteran Maranatha*, *5*(1), 33–48.

Wingfield, P. T. (2001). Protein Precipitation Using Ammonium Sulfate. *Current Protocols in Protein Science*, *13*(1), 1–10. https://doi.org/10.1002/0471140864.psa03fs13

Yanti, Ermawati, & Anas, E. (2016). Perbedaan Kadar Superokside Dismutase pada Remaja dengan Dismenore Primer dan Tanpa Dismenore Primer. *Jurnal Kesehatan Andalas*, *5*(1), 120–124. https://doi.org/10.25077/jka.v5i1.454

Younus, H. (2018). Therapeutic Potentials of Superoxide Dismutase. *International Journal of Health Sciences*, *12*(3), 88–93.

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Degradation and stability testing of chloramphenicol ear drops using derivative spectrophotometry combined with chemometrics

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Abstract

Background: Chloramphenicol eye drops have a lower stability than solid dosage forms. Hence, it is necessary to assess their stability. One stability test that can be conducted is the forced degradation approach, which involves applying stress conditions that are more severe than those used in accelerated stability testing.

Objective: This study used forced degradation to explore the stability profile of chloramphenicol ear drops.

Method: Stability analysis was carried out using a derivative spectrophotometric instrument combined with chemometric analysis. The forced degradation study was conducted by exposing the sample to three conditions: acidic (0.1 N HCl at 80°C for 2 hours), alkaline (0.1 N NaOH at 80°C for 2 hours), and heat (90°C for 4 hours). Principal Component Analysis (PCA) and Partial Least Squares–Discriminant Analysis (PLS-DA) were utilized for the chemometric analysis.

Results: Sequential chloramphenicol observations with a zero to third derivative show a maximum wavelength of 278, 260, 234, and 292 nm. According to stability studies with forced degradation, chloramphenicol tended to degrade under alkaline and thermal conditions compared to acidic conditions. A typical grouping pattern amongst forced degradation treatments is revealed by chemometric analysis, which characterizes chloramphenicol's stability profile under different experimental settings.

Conclusion: The UV-Vis spectrophotometric approach, both non-derivative and derivative, can describe changes in chloramphenicol degradation profiles, although the specific degradation products generated remain unknown.

Keywords: Chloramphenicol, forced degradation, spectrophotometry, chemometrics, stability testing

1. Introduction

Streptomyces venezuelae is the source of the antibiotic chloramphenicol, which has a wide range of action and works against both Gram-positive and Gram-negative bacteria (Mitchell *et al*., 2015). It works through a bacteriostatic mechanism by penetrating bacterial cells and reversibly binding to the 50s ribosomal subunit (Singhal *et al*., 2020). On the market, chloramphenicol is available in various dosage forms, including solid preparations like tablets and capsules, semi-solid forms such as creams and ointments, and liquid preparations like suspensions, eye drops, and ear drops.

The Indonesian Pharmacopoeia Edition VI (Depkes, 2020) specifies that chloramphenicol ear drops must contain between 90.0 and 130.0% of the active ingredient, chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$), relative to the amount stated on the label. Due to their liquid nature, ear drops are generally less stable than solid dosage forms, so evaluating chloramphenicol's stability in ear drops is essential. Furthermore, the amide bond in chloramphenicol is susceptible to hydrolysis when exposed to acids, bases, or heat, which can compromise its stability. Additionally, the carbonyl group (C=O) in chloramphenicol is highly reactive with nucleophiles, leading to nucleophilic substitution

reactions with chlorine atoms (Cl) and resulting in degradation products such as 2-amino-1-(4-nitrophenyl)propane-1,3-diol (Al-Rimawi & Kharoaf, 2011).

Stability testing in the pharmaceutical field plays a crucial role in ensuring that a pharmaceutical preparation remains effective over time. A preparation is considered stable when its characteristics continue to meet established specifications, even after a period of storage or use. This stability is essential for the preparation to deliver optimal therapeutic effects. Therefore, conducting stability tests is vital to confirm that the quality of the preparation remains consistent with its initial standards, ensuring its efficacy. Additionally, stability testing helps determine the shelf life, appropriate storage conditions, and the best formulation methods for a pharmaceutical preparation, thereby preserving its stability (González-González *et al*., 2022).

Stability studies typically include real-time stability tests lasting 12 months and accelerated stability tests lasting 6 months. However, this study utilized a forced degradation approach for stability testing. This method subjects the test sample to more extreme conditions than those used in accelerated stability tests to determine stability characteristics and degradation pathways. The International Conference on Harmonisation (ICH) recommends specific degradation conditions, such as a 10°C increase in temperature (from 50 to 60°C), humidity levels exceeding 75%, and exposure to acid hydrolysis, base, oxidation, and photolysis. Forced degradation studies are essential for elucidating the stability characteristics and degradation pathways of an active pharmaceutical compound or preparation (González-González *et al*., 2022).

Derivative spectrophotometry, an advancement of traditional spectrophotometric methods, offers a viable alternative for stability testing through the forced degradation of chloramphenicol. According to Chadha & Bali (2016), this technique is noted for its accuracy in stability assessments and often enhances spectral resolution, resulting in more precise results. Furthermore, using UV-Vis spectrophotometry is justified by chloramphenicol's chromophore group, which absorbs UV light (Aisha *et al*., 2018; Lisnawati *et al*., 2019). In UV-Vis spectrophotometry, the intensity of absorbed light is measured and presented as absorbance data and spectra (Pratiwi & Nandiyanto, 2022). When conducting stability analysis with forced degradation, the spectrophotometric data can be visualized to create a more distinct grouping profile among treatments using chemometric analysis methods.

Chemometrics is a scientific discipline that employs mathematical and statistical techniques to process chemical data obtained from measurements. It facilitates data visualization and the extraction of relevant information. One significant application of chemometrics is in analyzing the degradation of drugs and pharmaceuticals. By using chemometric methods, researchers can create grouping profiles of degradation samples, allowing for an evaluation of the relationships between

different treatments and the effects of degradative conditions on the samples. Common techniques used in data exploration include Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA). The results from PCA and PLS-DA indicate the percentage of variance, reflecting the extent to which the information in the data can be explained (Roberto de Alvarenga Junior & Lajarim Carneiro, 2019).

Numerous studies have examined the stability of chloramphenicol, employing techniques such as chromatography and spectrophotometry. Research on the stability of chloramphenicol in eye drops and capsules utilized Thin Layer Chromatography (TLC), revealing degradation percentages of 23.75% in 1N HCl, 100% in 0.1N NaOH, and 24% under thermal conditions (Musharraf *et al*., 2012). These findings indicate that chloramphenicol experiences significant degradation in alkaline environments. Other studies have employed High-Performance Liquid Chromatography (HPLC) for stability testing, where samples are injected into a system, and the resulting peaks are analyzed (AlAani & Alnukkary, 2016). Additionally, further research utilized UV-Vis spectrophotometry to assess the stability of simvastatin compounds under forced degradation, evaluating results based on absorbance and degradation percentage (Chavhan & Ghante, 2014). Furthermore, UV-Vis spectrophotometry has been integrated with chemometrics in the stability testing of cefoxitin sodium (Attia *et al*., 2018).

Previous research reviews indicate a lack of studies focusing on the stability testing of chloramphenicol through forced degradation using derivative spectrophotometry and chemometric analysis. However, there have been some studies employing derivative spectrophotometry for stability tests on raw materials and various formulations, such as trandolapril (Jaiswal & Bali, 2024), dronedarone hydrochloride (Chadha & Bali, 2016), and colistin sulfate (Mutasim Elimam *et al.*, 2015). The specific stability of chloramphenicol has not been extensively investigated. This study aims to analyze the stability of chloramphenicol in ear drop preparations subjected to forced degradation under acidic, basic, and thermal conditions, utilizing derivative spectrophotometry in conjunction with chemometric analysis for evaluation.

2. Method

2.1. Instruments and materials

The instruments used in this study included a UV-Vis spectrophotometer (Shimadzu UV-1780, Japan) equipped with a UV Probe, a water bath, an analytical balance, a micropipette, a thermometer, and various glassware. The materials comprised standard chloramphenicol (Sigma-Aldrich) and chloramphenicol ear drops from brand XX, obtained from a pharmacy in Purwokerto,

which were still within their circulation period. Additional materials included 99.9% ethanol (Merck) and pro-analysis grade substances, such as 37% hydrochloric acid (HCl), 99% sodium hydroxide (NaOH), 30% hydrogen peroxide (H_2O_2), and distilled water.

2.3. Chloramphenicol measurement

The procedures conducted in this study included standard preparation, maximum wavelength measurement, and the development of a chloramphenicol standard curve, which modified methods from previous research on stability (Yugatama *et al*., 2019). The preparation of the chloramphenicol standard began by weighing 100 mg of chloramphenicol, which was then dissolved in 50% ethanol in a 100 mL volumetric flask to achieve a final concentration of 1000 µg/mL. This 1000 µg/mL stock solution was diluted with ethanol to obtain a 10 µg/mL concentration. The absorbance of the 10 µg/mL standard solution was measured using a UV-Vis spectrophotometer across the wavelength range of 200-400 nm to generate an absorption spectrum. The 1000 µg/mL stock solution was diluted in ethanol to a 100 µg/mL concentration to create the standard curve. This 100 µg/mL solution was then transferred to a 10 mL volumetric flask, and ethanol was added to achieve a final concentration series of 4-16 µg/mL. The absorbance of each solution in this series was measured using a UV-Vis spectrophotometer, with ethanol serving as the blank.

2.4. Evaluation of analysis parameters

To confirm the parameters for spectrophotometric analysis of chloramphenicol concentrations, we evaluated linearity, accuracy, and precision according to ICH Q2(R2) guidelines (Ermer, 2025). Linearity was assessed by measuring levels across seven different concentrations and analyzing the resulting standard curve data. Each concentration was tested in triplicate, with absorbance measured at the maximum wavelength using a spectrophotometer. The linearity was determined by calculating the correlation coefficient (r) through linear regression between concentration and measured absorbance. For accuracy testing, the standard addition method was employed. We added three concentrations of standard solution, 75, 100, and 125%, to the chloramphenicol sample solution, with each measurement conducted in triplicate. Absorbance values were recorded using a spectrophotometer. Additionally, we calculated the % recovery for accuracy and the % RSD and HORRAT values for precision testing, using both underivatized and derivatized spectra. The calculations for % recovery and % RSD were performed as follows:

$$\%\ recovery = \frac{\text{measured chloramphenicol concentration in sample}}{\text{added chloramphenicol concentration in sample}} \times 100\%$$

$$\%\ RSD = \frac{\text{standard deviation of chloramphenicol measurement results in sample}}{\text{mean of chloramphenicol measurement results in sample}} \times 100\%$$

2.5. Forced degradation test

The forced degradation test method carried out in this study was adapted from Vyas *et al*. (Vyas *et al*., 2023). Initially, 0.5 mL of chloramphenicol ear drops were placed in a 50 mL measuring flask, and 50% ethanol was added to the mark. From this solution, 15 mL was transferred to another 50 mL measuring flask, and ethanol was added to the mark, resulting in a final sample concentration of 30 µg/mL. 5 mL of the sample was placed in a test tube for the degradation test. To create acidic conditions, 1 mL of 0.1 N HCl was added; for basic conditions, 1 mL of 0.1 N NaOH was added; and for oxidation conditions, 1 mL of 30% H_2O_2 was added. The samples for acidic, basic, and thermal conditions were heated at 80°C for 2 hours, while the thermal condition sample, containing only 5 mL of the sample, was heated at 90°C for 4 hours using a water bath. After heating, the samples were cooled and neutralized if they had undergone acid or base treatment before being measured with a spectrophotometer. Subsequently, all samples from the test tubes were transferred into a 10 mL measuring flask, and the volume was adjusted with 50% ethanol. The absorbance of the sample solution was then measured using a spectrophotometer over a wavelength range of 200-400 nm, with 50% ethanol serving as the blank solution.

3. Results and discussion

3.1. Chloramphenicol UV-Vis spectral profile and standard curve

The absorption spectra profile of chloramphenicol in the UV region was obtained by first measuring a standard solution. After preparing the standard solution, the next step involved measuring the maximum wavelength using a UV-Vis spectrophotometer. This measurement aimed to determine the maximum analysis sensitivity at the maximum wavelength, and the absorbance changes reached their highest value for each concentration series (Apriliyani *et al*., 2018). The maximum wavelength of the chloramphenicol standard solution was measured using a spectrophotometer over a range of 200-400 nm. The results of this measurement are presented in **Figure 1**.

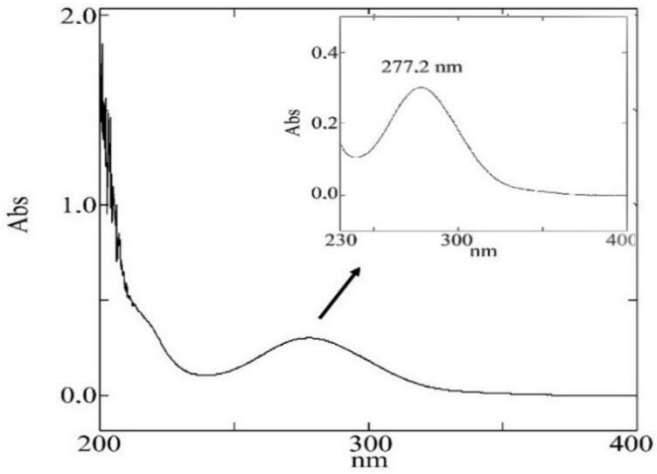

Figure 1. Absorption profile and maximum wavelength of chloramphenicol

The measured wavelength of chloramphenicol was found to be 277.20 nm. This aligns with the value stated in the Indonesian Pharmacopoeia Edition VI, which indicates that chloramphenicol has a wavelength of approximately 278 nm. The difference between the measured wavelength and the literature value falls within the acceptable tolerance limit of ±2 nm. The wavelength of 278 nm was then utilized to create a standard curve. This standard curve establishes the relationship between absorbance and the concentration of varying standard solutions at the maximum wavelength. A standard curve is deemed effective if there is a linear relationship between concentration and absorbance, indicating that an increase in concentration corresponds to an increase in absorbance. The results of the standard curve for the chloramphenicol standard solution are shown in **Figure 2**.

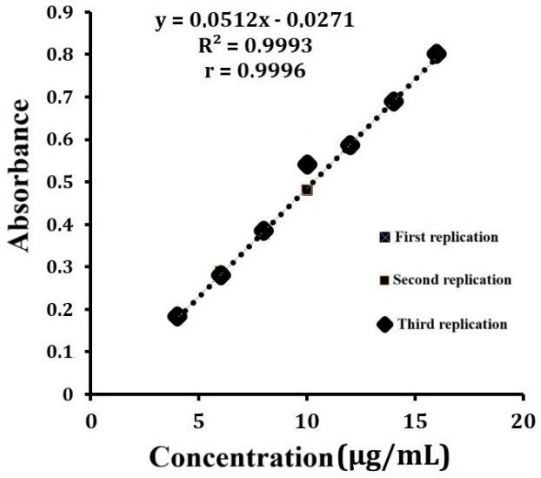

Figure 2. Standard curve of chloramphenicol standard solution
Each measurement concentration was replicated three times (n=3)

The standard curve results demonstrated the relationship between concentration and absorbance for the standard solution within the 4 to 16 µg/mL range, based on three measurements. Furthermore, the analysis of the standard curve data yielded a linear regression equation of y = 0.0512x - 0.0271, with a correlation coefficient (r) of 0.9996, confirming the linearity of the data.

3.2. Evaluation of analysis parameters

A linearity test was conducted using UV-Vis spectrophotometry to assess the detector's response to substance concentration variations and determine if the relationship is linear (Rohmah *et al*., 2021). Linearity is considered acceptable when the correlation coefficient falls between 0.995 and 1. The linearity results presented in the standard curve in Figure 2 indicate a correlation coefficient (r) of 0.9996. This result meets the established criteria, as the r value is within the range of $0.995 \leq r \leq 1$, demonstrating that the method has good linearity and can produce a linear response.

Accuracy testing measures how closely the analysis results align with the analyte concentration, expressed as percentage recovery (% recovery). In contrast, precision testing evaluates the method's repeatability by assessing the consistency of results across multiple repetitions, indicated by the percentage of Relative Standard Deviation (% RSD) (Ehling *et al*., 2025). Both accuracy and precision are determined using the standard addition method, which involves adding standard solutions at three concentration levels: 75, 100, and 125% to the sample solution. Measurements are then taken using a spectrophotometer, with three replicates for each concentration. The results of the accuracy and precision tests, calculated as percentage recovery (% recovery) and percentage of relative standard deviation (% RSD), are presented in **Table 1**.

Table 1. Test results for accuracy and precision

Concentration level	Replication	Standard added	Measured level	*Recovery* (%)	Average	SD	RSD (%)	HORRAT
75%	1	5.20	5.56	106.92	111.72	5.11	4.57	0.37
	2	5.20	6.09	117.11				
	3	5.20	5.78	111.15				
100%	1	6.96	7.72	110.91	111.68	1.58	1.41	0.12
	2	6.96	7.90	113.50				
	3	6.96	7.70	110.63				
125%	1	8.73	9.81	112.37	111.07	2.97	2.67	0.23
	2	8.73	9.88	113.17				
	3	8.73	9.40	107.67				

The percentage recovery value for a reliable accuracy test, according to ICH guidelines, should fall within the range of 80–120%. Precision is considered acceptable if the HORRAT (Horwitz

Ratio) is calculated from the observed RSD and the predicted RSD is less than 2 (Ehling *et al.*, 2025). The results presented in **Table 1** for both accuracy and precision tests demonstrate that the applied analytical method meets these criteria. Specifically, the accuracy test results at concentration levels of 75, 100, and 125% yielded values of 111.72% ± 5.11, 111.68% ± 1.58, and 111.07% ± 2.97, respectively. These values are within the acceptable 80–120% range, indicating good accuracy. Additionally, the precision results based on the % RSD for the same concentration levels were 4.57, 1.41, and 2.67%, with corresponding HORRAT calculations of 0.37, 0.12, and 0.23, all below 2. This confirms that the method also has adequate precision (Ehling *et al*., 2025).

3.3. Chloramphenicol derivatives spectrum

The spectrophotometric analysis data will be presented as absorbance values and UV spectra. Derivatization spectra in spectrophotometric measurements enhance spectral resolution without requiring compound separation, achieved by calculating derivative data from the UV spectra (Redasani *et al*., 2018). In this study, chloramphenicol absorption spectra were derivatized by first measuring a standard chloramphenicol solution across a concentration range of 4-16 µg/mL to establish a standard curve. The derivatization process continued until the third derivative was reached. The results of this derivatization process for chloramphenicol absorption spectra are illustrated in **Figure 3** and summarized in **Table 2** below.

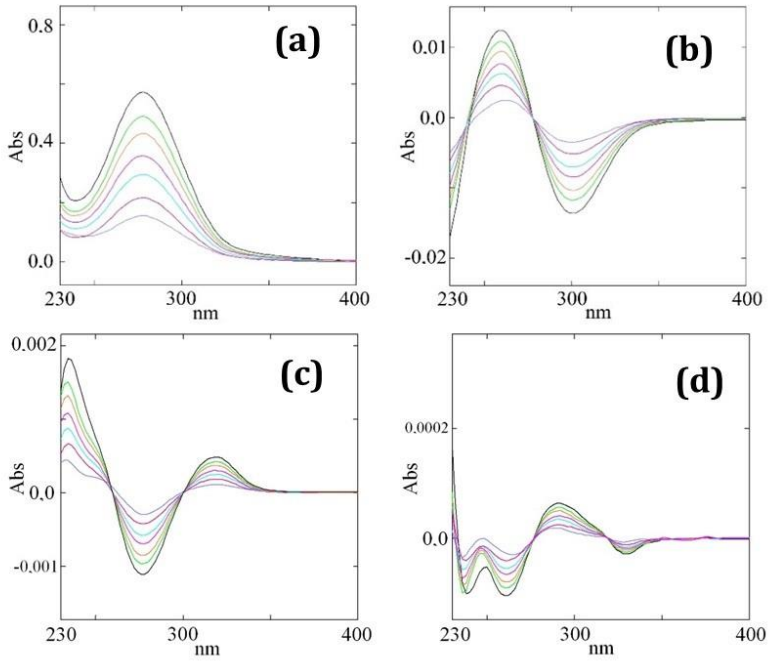

Figure 3. Spectra of chloramphenicol underivatives (a), first (b), second (c), and third (d) derivatives

Table 2. Regression analysis of data accuracy and precision in derivative spectra measurements

Parameter	D_0	D_1	D_2	D_3
λ_{max}	278 nm	260 nm	234 nm	292 nm
Slope	0.0347	0.0008	0.0001	0.000004
R	0.9993	0.9985	0.9981	0.9980
% RSD	5.661	7.488	7.820	6.926
HORRAT	0.48	0.64	0.68	0.60
% *Recovery*	111.727	123.647	131.4655	130.5077

The results presented in **Figure 3** and **Table 2** indicate that the maximum wavelengths for the zero to third derivatives were obtained sequentially: 278, 260, 234, and 292 nm. These values correspond to the sizes of the absorption peak amplitudes in the spectrum. Furthermore, the linearity met the acceptance criteria, with the correlation coefficient (r) falling within the range of reasonable linearity requirements, specifically $0.995 < r < 1$ (Ermer, 2025).

The % RSD values for the zero to third derivatives were 5.661, 7.488, 7.820, and 6.926%, with corresponding HORRAT values of 0.48, 0.64, 0.68, and 0.60, indicating adequate precision (Ehling *et al*., 2025). The % recovery for the zero to third derivatives was recorded at 111.727, 123.647, 131.465, and 130.507%. Although there was a tendency for an increase in % recovery during derivatization (**Table 2**), ANOVA analysis revealed no significant differences among these values. Therefore, measurements from the zero to third derivative spectra yield relatively consistent results.

3.4. Forced degradation test

Samples treated with acid, base, oxidation, and thermal methods and control samples without treatment were scanned using a spectrophotometer in the wavelength range of 200-400 nm. The resulting spectra for these samples are shown in **Figure 4**, which illustrates the effects of the various treatments on chloramphenicol.

The degradation of chloramphenicol is evident from the differences in the spectra of control and treatment samples. The spectra for the acid, base, and thermal samples still show analyzable peaks. In contrast, the oxidation sample produced a noisy spectrum in the 230-300 nm wavelength range, which corresponds to the absorption area of chloramphenicol, complicating analysis. Consequently, further analysis was conducted only on the samples treated with acid, base, and thermal methods.

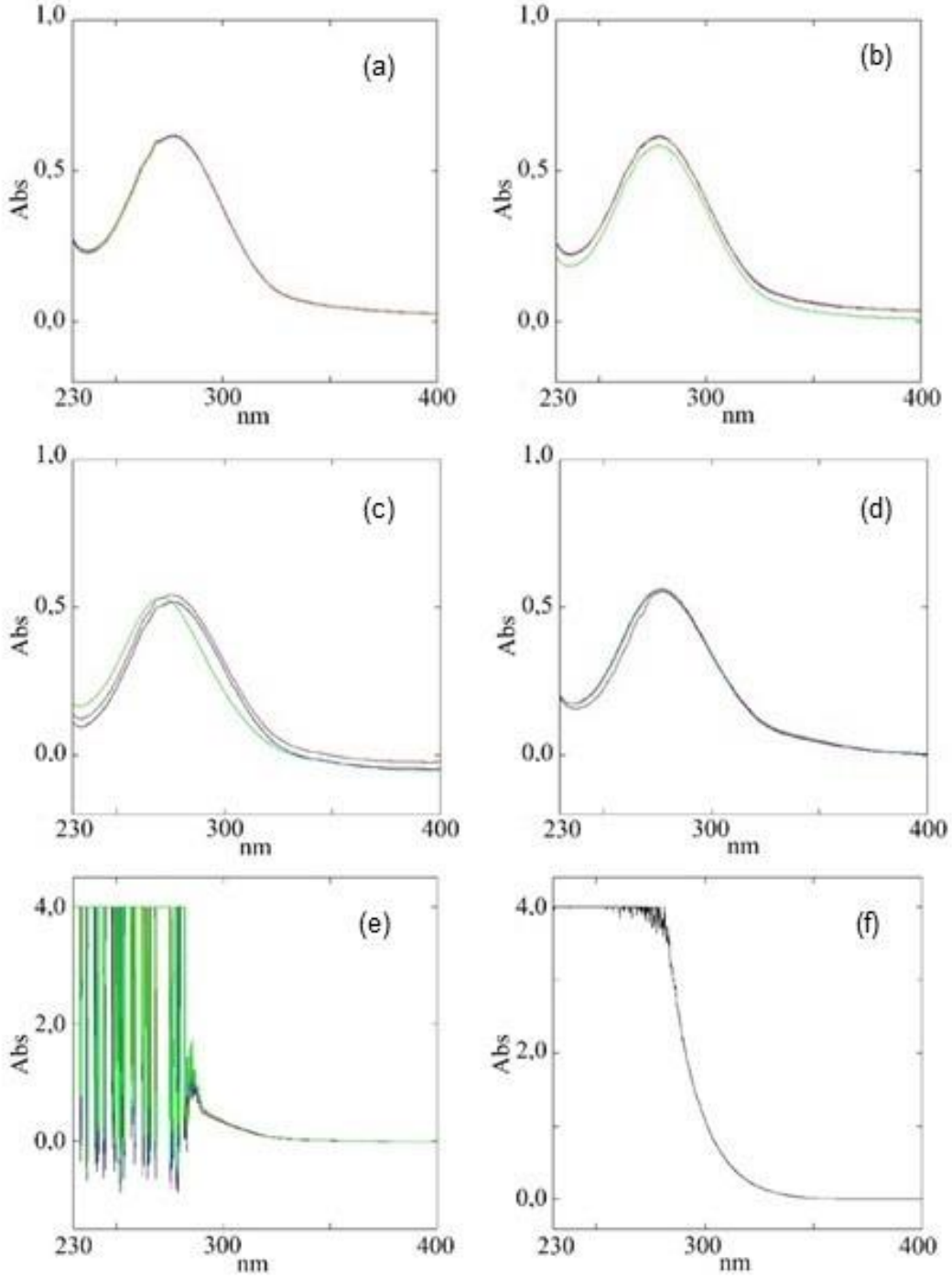

Figure 4. UV-Vis spectrum of forced degradation of chloramphenicol control sample (a), acid (0.1 N HCl; 80°C for 2 hours) (b), base (0.1 N NaOH; 80°C for 2 hours) (c), thermal (90°C for 4 hours) (d), oxidation (30% H_2O_2; 80°C for 2 hours) (e), absorbance of H_2O_2 solvent (f)
Measurements were carried out with three replications (n=3)

The degradation spectrum was analyzed using chemometric techniques, specifically PCA and PLS-DA. Chemometric analysis provides a percentage of variance that indicates how much information is retained and explained and how much is lost and cannot be accounted for. While there are no strict rules regarding variance limits, a minimum value of 50% is recommended, as it is considered sufficient to represent the data as a whole (Kamil & Hananto, 2023). Consequently, PCA and PLS-DA effectively characterize spectral data (Roberto de Alvarenga Junior & Lajarim Carneiro, 2019). The results of this analysis are presented in **Figure 5** for PCA and **Figure 6** for PLS-DA.

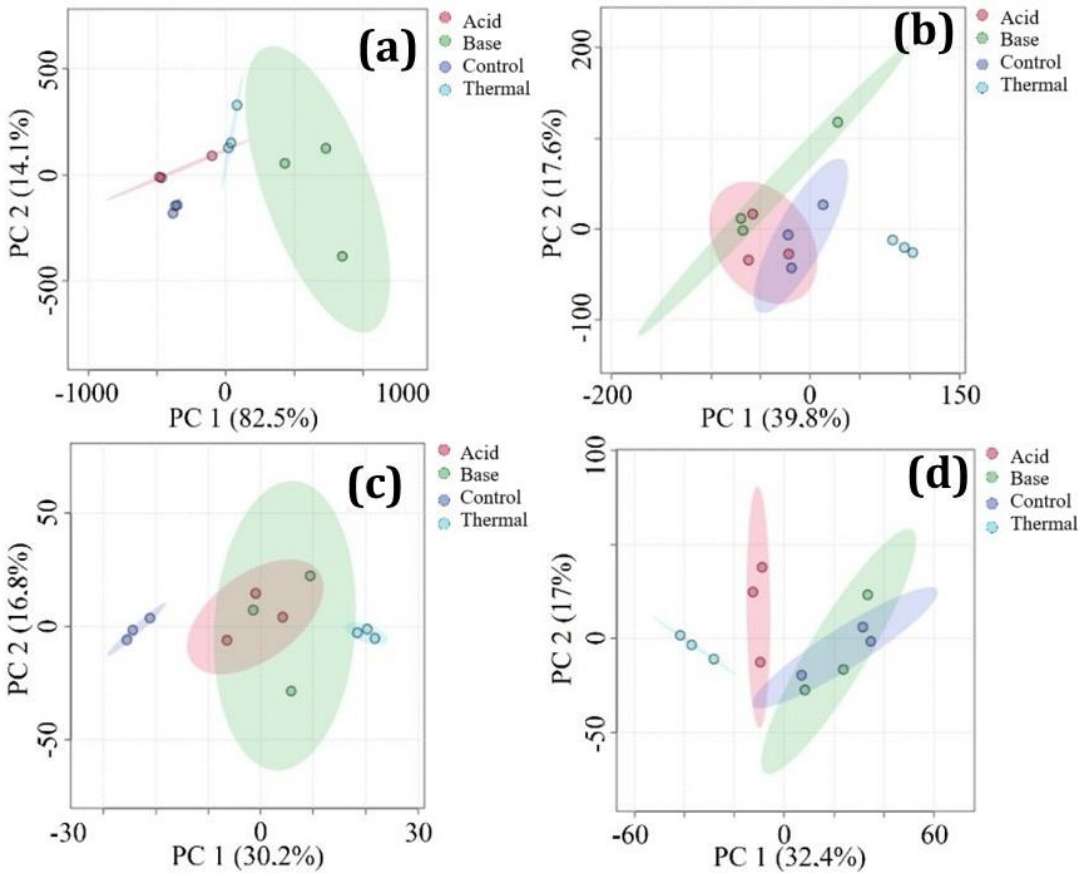

Figure 5. PCA results of the chloramphenicol spectrum of the zero (a), first (b), second (c), and third (d) derivatives

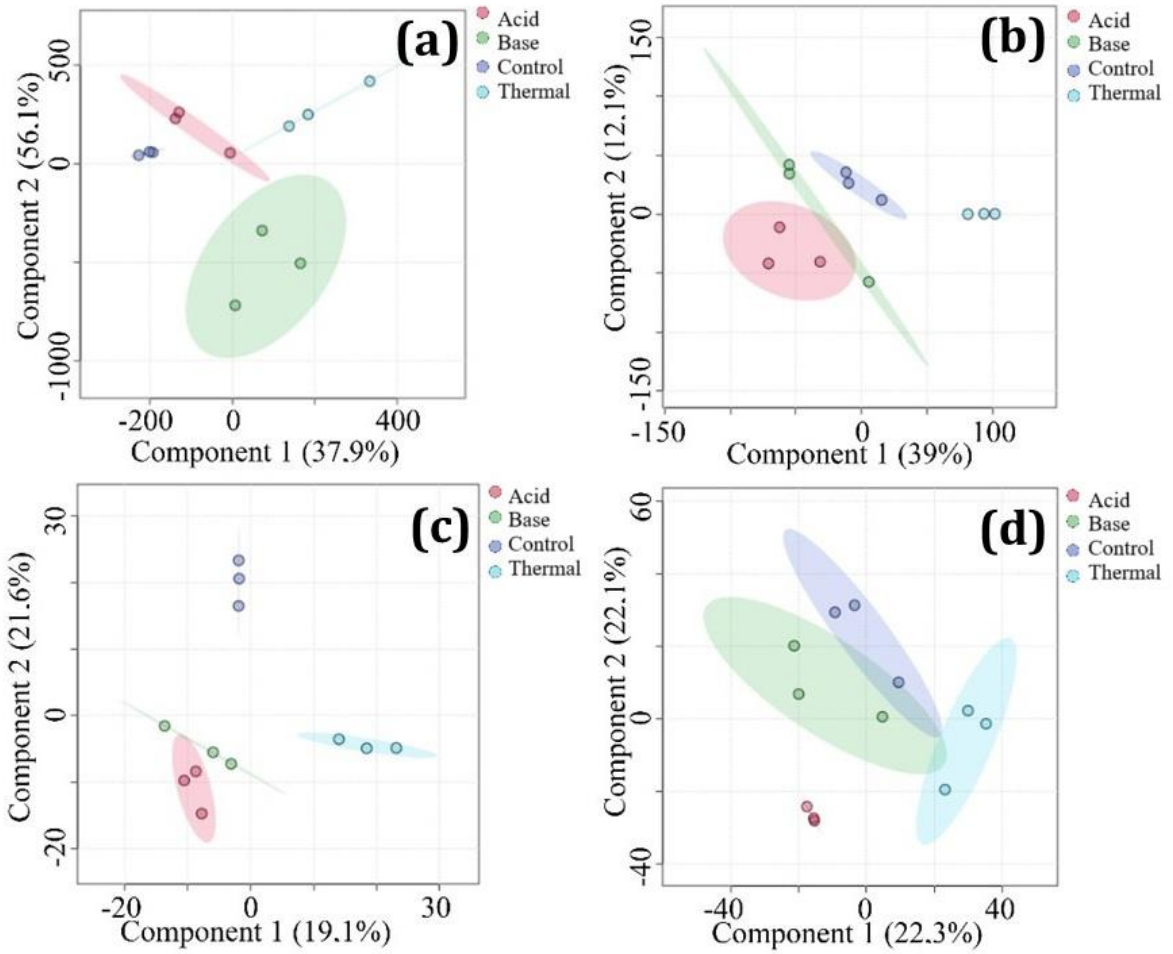

Figure 6. PLS-DA results of the zero (a), first (b), second (c), and third (d) chloramphenicol derivative spectra

In chemometrics, PCA (Principal Component Analysis) simplifies data by creating new variables, known as Principal Components (PC), which are linear combinations of the original variables. The results of this analysis are illustrated in **Figure 5**, which displays the grouping profile of treatment and control samples. Notably, the acid group tends to cluster closely with the control group. On the X-axis (PC1), the first principal component represents the direction in high-dimensional data space that accounts for the most significant variation. The Y-axis (PC2) indicates the second principal component, which captures the second most significant variation in the data, ensuring it is orthogonal to PC1. The numbers in brackets next to the axis labels denote the percentage of data variance explained by each component. The combined variance explained by the first two PCs for non-derivative data is 96.6%, which decreases during derivatization. The first derivative results yield a variance of 57.4% for the first two PCs, maintaining above 50%.

The following chemometric analysis utilizes PLS-DA, a method for discrimination and classification that optimizes the separation between sample groups (Aminu & Ahmad, 2020). The

PLS-DA results, illustrated in **Figure 6**, demonstrate that the samples are closely clustered together. The variance explained by the first two components is 94.0% for the zero derivative, which decreases upon derivatization. Notably, a variance value exceeding 50% is only achieved in the first derivative chemometric results, reaching 51.1%. Both PCA and PLS-DA analyses provide insights into the forced degradation profiles under various conditions, as shown by the visualization of spectrum data, which illustrates the sample grouping following degradative treatments such as acid, base, and thermal exposure.

Figure 7 presents the results of the degradation percentages. The data indicates that chloramphenicol degradation is minimal under acidic conditions, based on both the derivatization spectrum and non-derivatives. In contrast, degradation is more significant under basic and thermal conditions. These findings align with previous research by Musharraf *et al.* (Musharraf *et al*., 2012)**,** which demonstrated that the least degradation occurred in samples tested under acidic conditions using TLC-densitometry.

Under basic and thermal conditions related to zero and second derivatives, the basic group generally exhibits a higher percentage of degradation than the thermal group. Conversely, thermal degradation outweighs basic degradation for the first and third derivatives. These findings align with previous research (AlAani & Alnukkary, 2016), which conducted stability tests involving forced degradation on chloramphenicol using HPLC. Their results also indicated that the degradation percentage under thermal conditions was greater than under basic conditions. The degradation percentage is calculated by subtracting the sample content after degradation from the sample content before degradation and multiplying the result by one hundred percent. **Figure 7** illustrates how derivative spectrophotometry can elucidate the forced degradation analysis profile of chloramphenicol by revealing the percentage of degradation under various conditions. However, to achieve more reliable results, further research using more specific analytical methods, such as chromatography with mass spectrometry detectors, is necessary to analyze its compounds' degradation better.

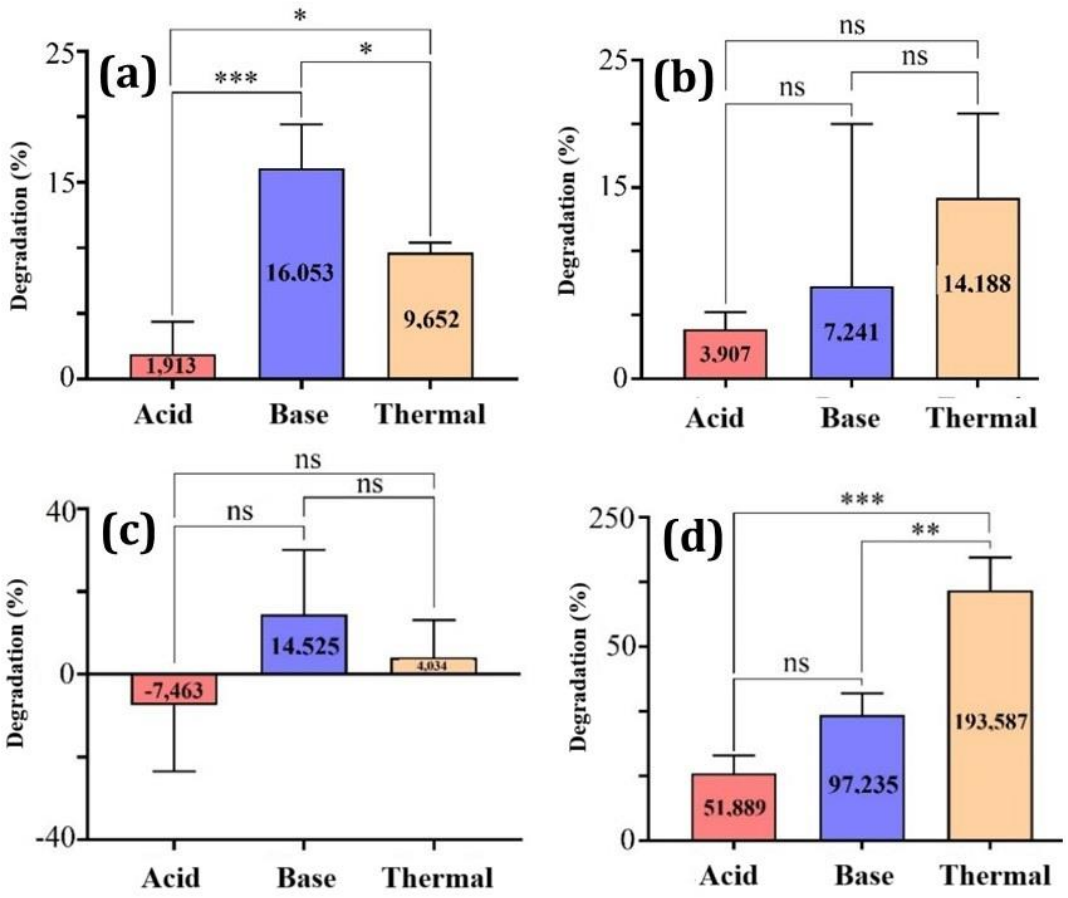

Figure 7. Degradation diagram of chloramphenicol derivatives to zero (a), first (b), second (c), third (d)

Previous studies have investigated chloramphenicol and its degradation products (AlAani & Alnukkary, 2016). The findings indicate that chloramphenicol is stable in acidic conditions, making it resistant to hydrolysis. In contrast, under basic conditions, particularly at pH levels above 10, chloramphenicol degrades to form 2-amino-1-(4-nitrophenyl)propane-1,3-diol, as these compounds are less stable in alkaline solutions. Additionally, amide compounds like chloramphenicol are thermolabile and can be easily hydrolyzed by water vapor when heated (Mitchell *et al*., 2015). The degradation of chloramphenicol is primarily attributed to the reactivity of its carbonyl carbon group (C=O) with nucleophiles, which facilitates hydrolysis reactions through nucleophilic substitution, leading to the formation of degradation products.

In line with other studies (AlAani & Alnukkary, 2016) reported that the degradation product of chloramphenicol, 2-amino-1-(4-nitrophenyl)propane-1,3-diol, has a wavelength of around 270 nm. This wavelength falls within the absorption range of chloramphenicol, which is 278 nm. Consequently, spectral overlap between the degradation products and the original chloramphenicol compound is more likely. Without a standard for the chloramphenicol degradation product, it is

challenging to assess this overlap using UV spectrophotometry accurately. The findings indicate that to effectively identify the degradation products formed, a standard for the chloramphenicol degradation product is essential, along with an analytical instrument capable of separating compounds, such as chromatography.

4. Conclusion

UV-Vis spectrophotometry methods, both non-derivative and derivative, can quantify the percentage of chloramphenicol degradation, although the formation of degradation products remains unclear. The degradation percentages obtained from the zero derivatives under acidic, basic, and thermal conditions are 1.913, 16.053, and 9.652%, respectively. The first derivative's percentages are 3.907, 7.241, and 14.188%. Additionally, the PCA and PLS-DA chemometrics analysis reveals the grouping profiles of forced degradation samples under various acidic, basic, and thermal conditions compared to the control. Notably, chloramphenicol in acidic conditions tends to cluster closer to the control sample. Furthermore, the variance from the PCA analysis for the zero and first derivatives was 96.6% and 57.4%, respectively. The PLS-DA analysis showed variances of 94.0% for the zero derivative and 51.1% for the first derivative.

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References

Aisha, S., Kuswandi, B., & Pratoko, D. K. (2018). Pengembangan Sensor Kloramfenikol Berbasis Bovine Serum Albumin menggunakan Spektrofotometri UV. *Pustaka Kesehatan*, *6*(1), 1-4. https://doi.org/10.19184/pk.v6i1.6609

AlAani, H., & Alnukkary, Y. (2016). Stability-Indicating HPLC Method for Simultaneous Determination of Chloramphenicol, Dexamethasone Sodium Phosphate and Tetrahydrozoline Hydrochloride in Ophthalmic Solution. *Advanced Pharmaceutical Bulletin*, *6*(1), 137–141. https://doi.org/10.15171/apb.2016.020

Al-Rimawi, F., & Kharoaf, M. (2011). Analysis of Chloramphenicol and Its Related Compound 2-Amino-1-(4-nitrophenyl)propane-1,3-diol by Reversed-Phase High-Performance Liquid Chromatography with UV Detection. *Chromatography Research International*, *2011*, 1–6. https://doi.org/10.4061/2011/482308

Aminu, M., & Ahmad, N. A. (2020). Complex Chemical Data Classification and Discrimination Using Locality Preserving Partial Least Squares Discriminant Analysis. *ACS Omega*, *5*(41), 26601–26610. https://doi.org/10.1021/acsomega.0c03362

Apriliyani, S. A., Martono, Y., Riyanto, C. A., Mutmainah, M., & Kusmita, K. (2018). Validation of UV-Vis Spectrophotometric Methods for Determination of Inulin Levels from Lesser Yam (*Dioscorea*

esculenta L.). *Jurnal Kimia Sains dan Aplikasi*, *21*(4), 161–165. https://doi.org/10.14710/jksa.21.4.161-165

Attia, K. A.-S. M., Abdel-Aziz, O., Magdy, N., & Mohamed, G. F. (2018). Development and Validation of Different Chemometric-Assisted Spectrophotometric Methods For Determination Of Cefoxitin-Sodium In The Presence of its Alkali-Induced Degradation Product. *Future Journal of Pharmaceutical Sciences*, *4*(2), 241–247. https://doi.org/10.1016/j.fjps.2018.08.002

Chadha, R., & Bali, A. (2016). Development and Validation of Stability-Indicating Derivative Spectrophotometric Methods for Determination of Dronedarone Hydrochloride. *Journal of Applied Spectroscopy*, *83*(2), 288–293. https://doi.org/10.1007/s10812-016-0283-4

Chavhan, V., & Ghante, M. (2014). Stability Indicating UV Spectrophotometric Method Development and Validation of Simvastatin In Bulk And Tablet Dosage Form. *Journal of Applied Pharmacy, 6*(2), 235 -246.

Depkes. (2020). *Farmakope Indonesia Edisi* VI. Departemen Kesehatan Republik Indonesia. Jakarta: Kementrian Kesehatan Republik Indonesia.

Ehling, S., Thompson, J. J., Schimpf, K. J., Pacquette, L. H., & Haselberger, P. A. (2025). A Contemporary Look At The Precision of Modern Analytical Methods In Food Analysis And The Relevance of The Horwitz Equation. *Journal of AOAC International*, qsaf026. https://doi.org/10.1093/jaoacint/qsaf026

Ermer, J. (2025). ICH Q2(R2): Validation of Analytical Procedures. In *Method Validation in Pharmaceutical Analysis* (351–372). John Wiley & Sons, Ltd. https://doi.org/10.1002/9783527831708.ch13

González-González, O., Ramirez, I. O., Ramirez, B. I., O'Connell, P., Ballesteros, M. P., Torrado, J. J., & Serrano, D. R. (2022). Drug Stability: ICH versus Accelerated Predictive Stability Studies. *Pharmaceutics*, *14*(11), 1-21. https://doi.org/10.3390/pharmaceutics14112324

Jaiswal, V. K., & Bali, A. (2024). Stability Indicating Derivative Spectrophotometric Method for Determination of Trandolapril in Bulk and in Formulation. *Journal of Applied Spectroscopy*, *90*(6), 1376–1383. https://doi.org/10.1007/s10812-024-01681-4

Kamil, F., & Hananto, A. (2023). Analysis Sentiment of Nestle Bear Brand during the Covid-19 Pandemic on Social Media Twitter. *Cakrawala Repositori IMWI*, *6*(1), 596–619. https://doi.org/10.52851/cakrawala.v6i1.166

Lisnawati, N., N.U, M. F., & Nurlitasari, D. (2019). Penentuan Nilai Spf Ekstrak Etil Asetat Daun Mangga Gedong Menggunakan Spektrofotometri UV - Vis. *Jurnal Riset Kefarmasian Indonesia*, *1*(2), 157–165. https://doi.org/10.33759/jrki.v1i2.35

Mitchell, S. M., Ullman, J. L., Teel, A. L., & Watts, R. J. (2015). Hydrolysis of Amphenicol and Macrolide Antibiotics: Chloramphenicol, Florfenicol, Spiramycin, and Tylosin. *Chemosphere*, *134*, 504–511. https://doi.org/10.1016/j.chemosphere.2014.08.050

Musharraf, S. G., Fatima, U., & Sultana, R. (2012). Stress Degradation Studies and Development of Stability-Indicating TLC-Densitometry Method For Determination of Prednisolone Acetate and Chloramphenicol In Their Individual and Combined Pharmaceutical Formulations. *Chemistry Central Journal*, *6*(7), 1-9. https://doi.org/10.1186/1752-153X-6-7

Mutasim Elimam, M., Wagiealla Shantier, S., Ahmed Gadkariem, E., & Awadalla Mohamed, M. (2015). Derivative Spectrophotometric Methods for the Analysis and Stability Studies of Colistin Sulphate. *Journal of Chemistry*, *2015*(1), 1-5. https://doi.org/10.1155/2015/624316

Pratiwi, R. A., & Nandiyanto, A. B. D. (2022). How to Read and Interpret UV-VIS Spectrophotometric Results in Determining the Structure of Chemical Compounds. *Indonesian Journal of Educational Research and Technology*, *2*(1), 1-20. https://doi.org/10.17509/ijert.v2i1.35171

Redasani, V. K., Patel, P. R., Marathe, D. Y., Chaudhari, S. R., Shirkhedkar, A. A., & Surana, S. J. (2018). A Review On Derivative UV-Spectrophotometry Analysis of Drugs In Pharmaceutical

Formulations and Biological Samples Review. *Journal of the Chilean Chemical Society*, *63*(3), 4126–4134. https://doi.org/10.4067/s0717-97072018000304126

Roberto de Alvarenga Junior, B., & Lajarim Carneiro, R. (2019). Chemometrics Approaches in Forced Degradation Studies of Pharmaceutical Drugs. *Molecules*, *24*(20), 1-24. https://doi.org/10.3390/molecules24203804

Rohmah, S. A. A., Muadifah, A., & Martha, R. D. (2021). Validasi Metode Penetapan Kadar Pengawet Natrium Benzoat pada Sari Kedelai di Beberapa Kecamatan di Kabupaten Tulungagung Menggunakan Spektrofotometer UV-Vis. *Jurnal Sains dan Kesehatan*, *3*(2), 120–127. https://doi.org/10.25026/jsk.v3i2.265

Singhal, K. K., Mukim, M. D., Dubey, C. K., & Nagar, J. C. (2020). An Updated Review on Pharmacology and Toxicities Related to Chloramphenicol. *Asian Journal of Pharmaceutical Research and Development*, *8*(4), 104–109. https://doi.org/10.22270/ajprd.v8i4.671

Vyas, A. J., Jadav, C. D., Patel, A. I., Patel, A. B., Shah, S. R., Sheth, D., & Dholakia, S. (2023). Review on Stability Indicating Assay Method or Forced Degradation Study: Strategy and Regulatory Consideration. *Asian Journal of Pharmaceutical Analysis*, *13*(2), 131–139. https://doi.org/10.52711/2231-5675.2023.00022

Yugatama, A., Nurmalinda, R., Rohmani, S., Ermawati, D. E., & Prihapsara, F. (2019). Effect of Temperature and Length of Storage on Chloramphenicol Eye Drop's Concentration. *IOP Conference Series: Materials Science and Engineering*, *578*(1), 1-5. https://doi.org/10.1088/1757-899X/578/1/012055

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Analysis of protein, fat, and iron in catfish and green spinach biscuits

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Abstract

Background: Stunting in Indonesia remains a significant health issue that cannot be overlooked. Stunting can be addressed through the consumption of foods derived from both animal and plant sources, which are high in protein and rich in essential amino acids beneficial for preventing stunting. The provision of biscuits fortified with catfish and spinach flour is anticipated to assist the government in reducing the prevalence of stunting.

Objective: The aim of this study was to develop a functional food product formula utilizing catfish and spinach flour, while also analyzing the nutritional content of the resultant biscuits.

Method: This research employed three biscuit formulas with varying ratios of catfish and spinach flour specifically F1 (15 g : 45 g), F2 (30 g : 30 g), and F3 (45 g : 15 g). Chemical analyses conducted on the biscuits included tests for moisture content and ash content using the gravimetric method, protein analysis utilizing the Kjeldahl method, fat analysis via the Soxhlet extraction method, and carbohydrate analysis employing the titrimetric method.

Results: Based on the tests of the three biscuit formulas, it was found that the biscuits with formula F3 meet the minimum requirements outlined by the SNI, except for ash content. The protein content was 11.03%, the fat content was 31.073%, the water content was 2.26%, and the ash content was 2.98%. The protein content in formula F3 was higher than that in formulas F1 and F2 and has a high enough iron content at 5.025 mg/100 g.

Conclusion: It can be concluded that biscuits with F3 formula meet the nutritional values required by SNI regarding protein, fat, and water content. This formula has an opportunity for further development to enhance the carbohydrate, iron, and ash content of the biscuit to comply with SNI standards.

Keywords: Catfish biscuit, food fortification, processed food, spinach biscuit, stunting

1. Introduction

Stunting remains a significant nutritional challenge for the Indonesian government. Stunting is a growth disorder that occurs during the early stages of a child's life and can result in permanent damage (UNICEF/WHO/World Bank Group, 2021). Furthermore, addressing stunting is one of the objectives of the Sustainable Development Goals (SDGs), specifically related to Goal 2: Zero Hunger, which aims to reduce stunting by 40% by 2025 (Kemenkes RI, 2020).

Stunting is caused by inadequate nutrient intake, particularly protein and iron, as well as limited knowledge among parents regarding nutrition and the preparation of nutritious foods. These factors adversely affect the nutritional intake of children from conception to birth (Handarini & Madyowati, 2021; Rahmawati *et al*., 2021). Stunting requires special attention and must be prevented, as it is associated with decreased intellectual abilities and productivity, as well as an increased risk of degenerative diseases in adulthood. The growth of brain cells, which occurs rapidly, typically ceases or reaches its peak by the age of 4-5 years. Consequently, optimal brain development can only be achieved if the child attains a good nutritional status (Mohamad *et al*., 2022).

Efforts to improve the nutritional status of toddlers can be made by developing supplementary feeding formulas to enhance their nutritional value. One form of supplementary food is biscuit, a dry food product that is processed by baking dough using wheat flour, fat, and leavening agents. Biscuit can be formulated by incorporating other permitted food items, such as local ingredients (Wijinindnyah *et al*., 2022). Utilizing local food sources can serve as an alternative to address limited access to food for families and help mitigate food insecurity and malnutrition (Sutyawan *et al*., 2022). Examples of local food ingredients from Kalimantan that can be processed into biscuits include catfish and green spinach. These ingredients are commonly consumed by the local population, readily available, and relatively affordable.

Food plays an important role in the growth of toddlers. Addressing malnutrition in toddlers can be done through the diversification and development of supplementary food formulas, considering aspects of nutrition, health benefits, acceptability, shelf-life, and the advantages of local food resources. Meeting the nutritional needs of toddlers can be achieved by increasing the intake of nutritious foods, such as catfish and spinach (Untari *et al*., 2022).

Catfish contains 17% protein, 6.6% fat, 0.23 – 1.3% carbohydrates, 0.9% ash, and 74.4% water. Compared to the fat content of other freshwater fish such as snakehead fish (4.0%) and carp (2.9%), catfish has a higher fat content (Nurfajrina & Hastuti, 2021). Spinach is rich in calcium, iron, vitamin A, B, E, and C, fiber, and beta-carotene. It also has high mineral content, especially iron, which can be utilized to combat stunting (Suciati *et al*., 2020).

Based on the background, the researchers conducted a study aimed at creating a functional food formula using catfish and spinach flour, also analyzing the macronutrient and iron content of the biscuits. With the development of this biscuit formula, we hope to create a functional food product that can contribute to stunting prevention efforts. Fortified biscuits made with catfish and spinach flour are expected to support government initiatives aimed at preventing stunting.

2. Methods

2.1. Sample preparation

The research was conducted at the Food Technology Laboratory and the Chemistry Laboratory of the Nutrition Departement, Poltekkes Kemenkes Palangka Raya. The research was conducted from January to March 2024. The sample preparation stage consists of making catfish flour, spinach flour, and the formulation of the biscuit.

2.1.1. Preparation of catfish flour

The procedure for processing catfish flour was based on the method used by Suciati *et al*. (2020), with slight modifications. One kilogram of cleaned catfish was weighed. The weighed catfish was seasoned with lime juice and salt and steamed for approximately 10 minutes. After that, to remove excess fat, the catfish was crushed and squeezed using cloth. The crushed catfish was dried in a dehydrator at 40°C for 10 hours. The dry catfish was ground using a blender, mixed with wheat flour until evenly mixed, and sifted with an 80 mesh sieves.

2.1.2. Preparation of spinach flour

The procedure for processing spinach flour followed the method used by Machfudloh *et al*. (2019) with modification. The spinach leaves were cleaned and separated from the stems. 300 grams of spinach leaves were dried using dehydrator at 60°C for 19 hours. The dried spinach leaves were ground using a blender for 5 minutes a sifted with an 80-mesh sieve.

2.1.3. Formulation of catfish and spinach biscuits

In this study, biscuits were made with three formulas and using 10 kinds of ingredients as shown in **Table 1** (Mohamad *et al*., 2022).

Table 1. Biscuit formulations

Ingredients	F1	F2	F3
Wheat flour	240 g	240 g	240 g
Catfish flour	15 g	30 g	45 g
Spinach flour	45 g	30 g	15 g
Milk powder	10 g	10 g	10 g
Margarine	50 g	50 g	50 g
Baking powder	3 g	3 g	3 g
Vanilla	3 g	3 g	3 g
Salt	3 g	3 g	3 g
Sugar	65 g	65 g	65 g
Egg	1	1	1

The procedure for making biscuits followed the method used by Suciati *et al*. (2020). All ingredients were weighed according to the formula of each biscuit and mixed thoroughly until well combined. The dough was then poured into a mold then baked in an oven at 100°C for 35 minutes.

2.2. Method and result analysis

The biscuits were analyzed for protein, carbohydrate, water, and ash content. These tests followed the method that used by Annisa & Suryaalamsah (2023), Bolang *et al*. (2022), Pebrina *et al*. (2021), Yudhistira *et al*. (2019) with minor modifications. Protein content was analyzed using Kjeldahl method, carbohydrates were measured using Titrimetric method, and fat content was

measured using Soxhlet extraction method. Water and ash content were analyzed using Gravimetric method. Iron content was assessed using Atomic Absorption Spectroscopy (AAS).

2.2.1. Protein analysis

As many as 2 grams of biscuit samples were placed in a 100 mL Kjeldahl flask, along with 2 catalyst tablets and some boiling stones. Then, 15 mL of H_2SO_4 (95%-97%), and 3 mL of H_2O_2 were slowly added, and the solution was allowed to stand for 10 minutes in an acid room. Destruction was performed at 410°C for about 2 hours until the solution became clear. Cooled the samples to room temperature and diluted with 50-75 mL of distilled water.

Afterward, 25 mL of 4% boric acid solution was added into an Erlenmeyer flask. The flask containing the destruction result was placed in a stem distillation apparatus, and 50-75 mL of sodium hydroxide thiosulfate solution was added. The distillate was collected in the Erlenmeyer flask containing the 4% boric acid solution until it reached a volume of at least 150 mL. The distillate was then titrated with standardized 0.2 N HCl until the color changed from green to neutral grey.

2.2.2. Fat analysis

An empty round-bottom flask was weighed for fat analysis. Two grams of biscuit sample were placed in a Soxhlet extractor and destructed at 60°C for 8 hours. The fat and chloroform mixture in the flask were evaporated until dry. The round-bottom flask containing the fat was placed in an oven at 105°C for 2 hours. The flask and fat were cooled in a desiccator for 30 minutes.

2.2.3. Carbohydrate analysis

Ten milliliters of biscuit sample solution was pipetted into an Erlenmeyer flask. Then, 10 mL of Fehling's A and B solution and 2-4 drops of 0.2% methylene blue were added. The mixture was heated on a hot plate magnetic stirrer. Once boiling, it was titrated with a standard sugar solution until the blue color disappeared. The titration was performed quickly.

2.2.4. Moisture content analysis

Two grams of finely ground biscuit sample were placed in a pre-weighed porcelain crucible. The sample was dried in an oven at 100-150°C (3-5 hours). After that, the sample was cooled in a desiccator and weighed. The sample was redried in the oven for 30 minutes, cooled, and weighed again. This process was repeated until a constant weight achieved.

2.2.5. Ash content analysis

An empty porcelain crucible was burned in a furnace at 550°C for 1 hour. The crucible was dried in an oven at 100-105°C for an hour, cooled in a desiccator for 15 minutes, and weighed until a constant weight was achieved. Next, 5 grams of biscuit were placed in the crucible and ash in the furnace at 550°C for 4 hours. The ash sample was dried in an oven at 100-105°C for an hour, cooled

in a desiccator for 15 minutes and weighed. The process was repeated until a constant weight was achieved.

2.2.6. Iron (Fe) analysis

The iron content of the biscuit samples was analyzed using Atomic Absorption Spectroscopy (AAS) with 510 nanometers wavelength.

3. Result and discussion

The nutritional content of the biscuits was tested using proximate analysis. The results of the analysis of the biscuits with catfish flour and spinach flour substitution are presented in **Table 2**.

Table 2. Results of moisture, ash, carbohydrate, protein, and fat content testing of formula F1, F2, and F3

No.	Chemical analysis	Results		
		F1 (mean ± SD)	F2 (mean ± SD)	F3 (mean ± SD)
1.	Protein (%)	9.74 ± 0.366	10.44 ± 0.087	11.03 ± 0.083
2.	Fat (%)	31.217 ± 0.253	30.939 ± 0.779	31.073 ± 0.512
3.	Carbohydrate (%)	43.08 ± 0.250	43.37 ± 0,193	43.13 ± 0,184
4.	Moisture content (%)	3.75 ± 0.013	2.08 ± 0.022	2.26 ± 0.017
5.	Ash content (%)	3.34 ± 0.029	2.93 ± 0.072	2.98 ± 0.057
6.	Iron (%)	7.157 ± 0.11	6.530 ± 0.08	5.025 ± 0.09

The protein content analysis for the three biscuit formulas, as presented in **Table 2**, shows that all biscuits have a protein content above 5%. According to SNI 2973-2011, the minimum protein requirement for biscuits is 5%. Protein is essential for the body to maintain normal metabolic processes. Both animal and plant proteins contain amino acids that help build and repair body tissues, form hormones and enzymes, and serve as an energy source (Sidoretno *et al*., 2022).

Insufficient consumption of protein-rich foods can lead to a protein energy deficiency in children (Untari *et al*., 2022). A fast-acting approach to addressing stunting is encouraging the continuous consumption of animal protein sources, which are high in protein and essential amino acids (Eliana *et al*., 2022). Fish, including catfish, is a rich source of amino acids (Handarini & Madyowati, 2021).

According to SNI 01-2973-1992, the minimum fat content required in biscuits is 9.5 g/ 100 g (Loppies *et al*., 2021). As shown in **Table 2**, all the biscuit formulas meet this standard, with fat content percentages exceeding 30%. Fat is necessary for the body to function properly and aids in the absorption of vitamins A, D, E, and K. A lack of fat can lead to vitamin deficiencies (Sidoretno *et al*., 2022). Research conducted by Pandiangan (2021) indicates that catfish contains omega-3 and omega-6 fatty acids, which are beneficial for human health, including children's health (Pandiangan, 2021). Fish contains omega-3 fatty acids, which are polyunsaturated essential fatty acids crucial for brain development in children (Handarini & Madyowati, 2021).

The carbohydrate content of biscuits with formulas 1, 2, and 3 are 43.08, 43.37, and 43.13%, respectively. According to SNI (1992), the minimum carbohydrate content required for biscuits is 70%, meaning that these biscuits do not meet the carbohydrate quality standards set by SNI. Carbohydrates serve as the body's primary energy source (Sidoretno *et al*., 2022). Ingredients in biscuits that contain carbohydrates include wheat flour, milk, and sugar (Gita & Danuji, 2018). To increase the carbohydrate content of biscuits, it is suggested to increase the proportion of wheat flour and powdered sugar in the biscuit dough. Wheat flour consists of 67-70% carbohydrates (Annisa & Suryaalamsah, 2023).

SNI 01-2973-2011 states that the maximum moisture content in biscuits should be 5%. The water content analysis for three biscuit formulas shows that all biscuits (F1, F2, and F3) have moisture levels below 5%. Moisture content is crucial for determining the freshness and shelf-life of food products. High moisture content promotes the growth of microorganisms, leading to faster spoilage (Sidoretno *et al*., 2022).

The ash content of the biscuit formulas, as presented in **Table 2**, exceeds the maximum allowed by SNI, which is 2%. Ash content measures the mineral content in food (Sidoretno *et al.*, 2022). The high ash content in these biscuits is influenced by the mineral-rich ingredients used, particularly spinach. Spinach contains high levels of minerals, such as calcium, iron, and vitamins A, B, E, and C (Suciati *et al*., 2020).

The average iron content found in the biscuits was as follows: formula F1 = 7.157 mg/ 100 g, formula F2 = 6.530 mg/ 100 g, and formula F3 = 5.025 mg/ 100 g. The biscuit formula with the highest iron content was F1. Based on The Nutritional Adequacy Rate for Indonesian People, the iron intake requirement is 7 – 8 mg per day (Kemenkes RI, 2019). Consuming 100 g of formula F1 biscuit can meet the iron requirement per day.

Green spinach is rich in iron, which is essential for red blood cell production. Adequate red blood cell production helps prevent anemia. Iron-deficiency anemia increases the risk of low birth weight (LBW), reduced iron stores in infants, and can disrupt brain growth and development (Sulistyaningrum & Fitriyanti, 2022). Fresh spinach contains 2.71 mg of iron per 100 grams (Falah AS *et al*., 2023). The higher iron content in the biscuits compared to fresh spinach may be due to the additional iron present in catfish. Catfish contains approximately 1.6 mg of iron per 100 grams (Sulistyaningrum *et al.*, 2021). Fish consumption can also help improve iron absorption (Handarini & Madyowati, 2021).

4. Conclusion

Based on nutritional calculations, it was found that biscuit with formula F3 met the minimum requirements of SNI regarding moisture, protein, and fat content. However, it did not meet the minimum standards of carbohydrate, ash, and iron content (except formula F1). Further research should modify the biscuit formula by adding the concentration of spinach flour in the formula and combining the formula with wheat flour, so that the biscuit can meet the minimum standards of carbohydrate, ash, and iron content.

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References

Annisa, S. N., & Suryaalamsah, I. I. (2023). Formulasi Cookies dari Tepung Hati Ayam dan Tepung Kedelai Sebagai Makanan Sumber Zat Besi Pencegah Anemia Pada Remaja Putri. *Muhammadiyah Journal of Nutrition and Food Science (MJNF)*, *4*(1), 14–27. https://doi.org/10.24853/mjnf.4.1.14-27

Bolang, A. S. L., Rizal, M., Nurkolis, F., Mayulu, N., Taslim, N. A., Radu, S., Samtiya, M., Assa, Y. A., Herlambang, H. A., Pondagitan, A. S., & Vivo, C. D. (2022). Cookies Rich In Iron (Fe), Folic Acid, Cobalamin (Vitamin B12), and Antioxidants: A Novel Functional Food Potential For Adolescent With Anemia. *F1000Research*, *10*(1075), 1–15. https://doi.org/10.12688/f1000research.74045.2

Eliana, Yuliantini, E., Kamsiah, & Yunianto, A. E. (2022). Pengaruh Pemberian Pangan Setengah Jadi Berbasis Ikan (Sosis Analog) dengan Kecukupan Protein Balita dalam Upaya Penurunan Stunting di Kabupaten Seluma. *Journal of The Indonesian Nutrition Association*, *45*(2), 173–182.

Falah AS, S., Aryani, A., & Ratnasari, I. (2023). Pengaruh Penambahan Bayam Hijau (*Amaranthus tricolor* L.) Terhadap Kualitas Gizi Dimsum Ikan Patin (*Pangasius* sp.). *Juvenil: Jurnal Ilmiah Kelautan Dan Perikanan*, *4*(1), 51–56. https://doi.org/10.21107/juvenil.v4i1.19025

Gita, R. S. D., & Danuji, S. (2018). Studi Pembuatan Biskuit Fungsional dengan Substitusi Tepung Ikan Gabus dan Tepung Daun Kelor. *BIOEDUSAINS: Jurnal Pendidikan Biologi Dan Sains*, *1*(2), 155–162. https://doi.org/10.31539/bioedusains.v1i2.323

Handarini, K., & Madyowati, S. O. (2021). Penyuluhan Gizi Seimbang dan Olahan Pangan Lokal Pencegah Stunting Pada Ibu Paud Di Surabaya. *Jurnal Karya Abadi*, *5*(3), 507–514.

Kemenkes RI. (2019). *Peraturan Menteri Kesehatan Republik Indonesia Nomor 28 Tahun 2019 Tentang Angka Kecukupan Gizi Yang Dianjurkan Untuk Masyarakat Indonesia*. Jakarta: *Kementerian Kesehatan Republik Indonesia*.

Kemenkes RI. (2020). *Surat Edaran Nomor: HK.02.02/IV/2243/2020 Tentang Pemanfaatan Obat Tradisional Untuk Pemeliharaan Kesehatan Pencegahan Penyakit, Dan Perawatan Kesehatan*. Jakarta: *Kementerian Kesehatan Republik Indonesia*.

Loppies, C. R. M., Soukotta, D., & Gaspersz, F. F. (2021). Komposisi Gizi Biskuit Dengan Substitusi Konsentrat Protein Ikan (KPI). *Prosiding Simposium Nasional VIII Kelautan Dan Perikanan*, 359–367.

Machfudloh, M., Awaliyah, I. N., & Takwanto, A. (2019). Pengaruh Suhu Spray Drying Dan

Penambahan Maltodextrin Terhadap Aktivitas Antioksidan (IC_{50}) Pada Bayam Hijau (*Amaranthus hybridus* L.). *Distilat*, *5*(2), 52–57.

Mohamad, F., Setiawan, D. I., Slamet, N. S., Sapiun, Z., & Pomalingo, A. Y. (2022). Potensi Biskuit "TYam"(Biskuit Dengan Substitusi Tepung Tempe dan Serbuk Bayam) Sebagai Alternatif Pencegahan Stunting Pada Balita. *Journal Health & Science : Gorontalo Journal Health and Science Community*, *6*(1), 51–61. https://doi.org/10.35971/gojhes.v5i3.13797

Nurfajrina, A. A., & Hastuti, W. (2021). Formulasi Tepung Mocaf dan Tepung Ikan Patin Terhadap Kualitas dan Nilai Gizi Cookies Mocaf Patin. *JGK: Jurnal Gizi Dan Kesehatan*, *1*(2), 95–103. https://doi.org/10.36086/jgk.v1i2.1087

Pandiangan, M. (2021). Penentuan Komposisi Asam Lemak pada Minyak Ikan Patin. *Jurnal Riset Teknologi Pangan Dan Hasil Pertanian (RETIPA)*, *1*(2), 76–82. https://doi.org/10.54367/retipa.v1i2.1210

Pebrina, S. M., Wahyuni, S., & Susilowati, P. E. (2021). Karakteristik Fisik Biskuit Formulasi Tepung Wikau Maombo Dengan Tepung Spirulina Plantesis. *Jurnal Sains Dan Teknologi Pangan*, *6*(1), 3718–3730.

Rahmawati, S., Wulan, A. J., & Utami, N. (2021). Edukasi Pemberian Makanan Pendamping Asi (MPASI) Sehat Bergizi Berbahan Pangan Lokal Sebagai Upaya Pencegahan Stunting di Desa Kalisari Kecamatan Natar Lampung Selatan. *Jurnal Pengabdian Masyarakat Ruwai Jurai*, *6*(1), 47–50.

Sidoretno, W. M., Wardaniati, I., Siagian, D. S., & Nuraini. (2022). Analisis Kadar karbohidrat, Lemak dan Protein pada Biskuit dengan Fortifikasi Tepung Tulang Ikan Patin sebagai Penanggulangan Stunting. *INCH : Journal of Infant And Child Healthcare*, *1*(2), 57–62.

Suciati, G., Karsiwi, R. R. M., & Gusnadi, D. (2020). Biskuit Berbasis Ikan Patin Sebagai MPASI Bayi Usia 6-24 Bulan. *E-Proceeding of Applied Science*, *6*(2), 2188–2197. https://openlibrarypublications.telkomuniversity.ac.id/index.php/appliedscience/article/view/12015

Sulistyaningrum, H., & Fitriyanti, A. R. (2022). Kadar Hb dan Perubahan Berat Badan Tikus Wistar Model Anemia Defisiensi Besi Setelah Pemberian Minyak Ikan Patin. *Jurnal Gizi*, *11*(2), 77–84. https://doi.org/10.26714/jg.11.2.2022.77-84

Sulistyaningrum, H., Swastawati, F., Mexitalia, M., & Noer, E. R. (2021). Catfish Oil (*Pangasius hypophthalmus*) Effect To Ferritin and sTfR In Iron Deficiency Anemia. *Jurnal Gizi Indonesia (The Indonesian Journal of Nutrition)*, *10*(1), 28–35. https://doi.org/10.14710/jgi.10.1.28-35

Sutyawan, Novidiyanto, & Wicaksono, A. (2022). Optimalisasi Pemanfaatan Pangan Lokal yang Aman dan Bergizi dalam Upaya Pencegahan Stunting di Desa Ibul Kabupaten Bangka Barat. *Jurnal Panrita Abdi*, *6*(3), 565–577.

UNICEF/WHO/World Bank Group. (2021). Levels and Trends In Child Malnutrition. *World Health Organization*, 1–32. https://www.who.int/publications/i/item/9789240025257

Untari, D. S., Wibowo, T. A., Anwar, R., & Febriyanti, T. L. (2022). Diversifikasi Pengolahan Ikan Patin (*Pangasius pangasius*) Menjadi Nugget Dengan Konsep Zero Waste Sebagai Upaya Pemenuhan Gizi Anak. *Jurnal Pengabdian Pada Masyarakat*, *2*(2), 923–927.

Wijinindnyah, A., Selvia, J., Chotimah, H., & Gaol, S. E. L. (2022). Potensi Tepung Daun Kelakai (*Stenochlaena palutris* (Burn.f) Bedd) Pretreatment Asam sebagai Alternatif Pencegah Stunting. *Amerta Nutrition*, *6*(1SP), 275–282. https://doi.org/10.20473/amnt.v6i1sp.2022.275-282

Yudhistira, B., Sari, T. R., & Affandi, D. R. (2019). Karakteristik Fisik, Kimia dan Organoleptik Cookies Bayam Hijau (*Amaranthus tricolor*) dengan Penambahan Tomat (*Solanum lycopersicum*) sebagai Upaya Pemenuhan Defisiensi Zat Besi pada Anak-Anak. *Warta Industri Hasil Pertanian*, *36*(2), 83–95. https://doi.org/10.32765/wartaihp.v36i2.5286

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Determination of specific and non-specific parameters of saluang belum (*Luvunga sarmentosa* (Blume) Kurz.) root extract and quantification of its total flavonoid content

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Abstract

Background: Saluang belum (*Luvunga sarmentosa* (Blume) Kurz.) is an endemic plant native to the Kalimantan region. The Dayak people traditionally consume a decoction of *L. sarmentosa* root to improve stamina. Previous research has confirmed that the roots of *L. sarmentosa* possess antioxidant properties.

Objective: This study aims to determine the characterization values of both specific and nonspecific parameters of the ethanol extract from *L. sarmentosa* roots, as well as to assess the total flavonoid content.

Method: The research samples were collected from Timpah Village, Central Kalimantan. The methods for standardization employed the general standard parameters for Indonesian Herbal Extracts and the Pharmacopoeia. Specific parameters include the characteristics of the extract, phytochemical screening, and thin-layer chromatography profiles, while nonspecific parameters consist of determining water content, total ash, acid-insoluble ash content, and heavy metal contaminants (Pb, Cd, and Hg). The total flavonoid content was determined using a colorimetric method with $AlCl_3$ and quercetin as the standard.

Results: The extract was characterized as a thick, yellowish-brown substance with a strong, distinct odor and a bitter taste. It contains alkaloids, flavonoids, tannins, terpenoids, and steroids. The TLC profile revealed four distinct spots using a non-polar eluent and six spots with a polar eluent. The extract yield was 8.57%, with an average water content of 2.43±0.15%, total ash content of 1.50%, and acid-insoluble ash content of 1.00±0.87%. Heavy metal contamination levels were determined as follows: Pb < 0.001 mg/kg, Cd 0.446 mg/kg, and Hg 2.077 mg/kg. The total flavonoid content of the extract was quantified at 6.9147 ± 0.0083 mg QE/g extract.

Conclusion: All specific and non-specific parameters of the extract meet the requirements except for the heavy metal contaminants of Hg and Cd, which are still within the threshold limits set by BPOM. Meanwhile, the total flavonoid content measured was 6.9147 ± 0.0083 mg QE/g.

Keywords: Saluang belum, *Lavanga sarmentosa*, total flavonoid, Dayak tribe, extract standardization

1. Introduction

Indonesia is one of the countries with a tropical climate, allowing it to possess a diversity of advantageous biological natural resources. It is home to an abundance of plant species with immense potential as sources of medicinal compounds. Ancient civilizations have utilized medicinal plants for therapeutic purposes, drawing upon empirical knowledge. One of the plants commonly used for medicinal purposes is saluang belum (Marjoni, 2022).

Saluang belum (*L. sarmentosa*) is an endemic plant species found exclusively in Central and South Kalimantan. Local communities have historically recognized and employed it as traditional medicine for various of diseases, including back and kidney pain, as well as vitality enhancer (Anggriani, 2018). The Dayak people traditionally consume boiled water infused with the roots of *L. sarmentosa* once daily to enhance male fertility, sexual desire, and endurance (Wardah & Sundari, 2019). Previous studies have established that saluang belum root has antioxidant properties with an

IC_{50} of 80.33 ppm. It is undeniably impacted by the concentration of secondary metabolites, including phenol and flavonoid compounds (Wathan & Rizki, 2020). The highest total flavonoid content in *L. sarmentosa* roots was found in the extract using 96% ethanol as the solvent, measuring 6.56 ± 0.006 % w/w QE, compared to 4.10 ± 0.084 % w/w QE with methanol and 5.36 ± 0.012 % w/w QE with ethyl acetate (Wathan *et al*., 2023). According to the research by Musfirah *et al*. (2016), histopathological examinations of testicular organs treated with a 70% ethanol extract of *L. sarmentosa* roots indicate that spermatogenesis occurs normally in mice and that administration of the extract does not harm the testes. Applying a 70% ethanol extract of *L. sarmentosa* influences the formation of spermatocyte and spermatid cells, which indicates an increase in male fertility.

It is imperative to evaluate the character of traditional medicine with respect to its safety, effectiveness, and overall quality (Aulani, 2018). The regulatory framework governing the quality of traditional medicine is specified in regulation No. 32 of 2019, which is issued by the Indonesian Food and Drug Monitoring Agency (BPOM RI). Two parameters are used to standardize extracts, namely specific and nonspecific parameters (Najib *et al.*, 2017). Specific parameters refer to the aspect of both qualitative and quantitative chemical analysis that pertain to the concentrations of active compounds and the pharmacological activity of an extract. Nonspecific parameters refer to the physical, chemical, and microbiological analyses pertaining to the stability and safety of an extract (Marpaung & Septiyani, 2020). Determining the parameters of extract of *L. sarmentosa* is essential for ensuring consistent quality, efficacy, and safety, especially for its future development as a pharmaceutical product. This research highlights the importance of creating standardized parameters that can help validate and optimize the extract's therapeutic potential, supporting its reliable use in medicine. Determining total flavonoid content in *L. sarmentosa* extracts is critical for guaranteeing consistent extract quality and supporting its development as a standardized herbal medicine.

2. Method

2.1. Sample preparation

The roots of *L. sarmentosa* were collected in January 2023 from Timpah Village in Kapuas Regency, Central Kalimantan Province. The roots were extracted from mature *L. sarmentosa* plants, approximately 2 meters in height, exhibiting acceptable conditions, and identified as "male" due to the presence of thorns on the stem and leaf axils. A two kilograms sample was gathered, and the complete plant was submitted for identification to the Laboratorium Dasar at the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University.

2.2. Extraction

The fresh *L. sarmentosa* roots were wet-sorted and then thoroughly washed under flowing water and chopped. Subsequently, it was desiccated at 55°C in a drying cabinet. Using a blender, the root simplicia was subsequently ground into powder form (Kemenkes RI, 2017). The extraction of *L. sarmentosa* roots was performed using the maceration technique. A total of 250 g of *L. sarmentosa* root powder (simplicia) was weighed and transferred into a maceration vessel. An ethanol solvent was then added to cover the powder, reaching a depth of 1 to 2 cm above its surface. The extraction process was carried out over three cycles, each lasting 24 hours, with agitation every six hours. The solvent was replaced after each 24-hour period. The resulting macerate was filtered and evaporated using a water bath (50°C) to complete the extraction procedure.

2.3. Method and result analysis

2.3.1. Specific parameters

a) Extract description

The five senses describe the shape, color, odor, and flavor of the extract. It aims to provide an objective and straightforward introduction to the extract (Depkes RI, 2000). The shape, color, odor, and flavor of the extract can be described to a group of panelists.

b) Yield percentage

The yield is calculated as the proportion of the extracted substance to the initial simplicial (Depkes RI, 2000). The following formula is utilized to determine the percentage yield.

$$\% \text{ yield} = \frac{\text{weight of extract obtained (g)}}{\text{weight of simplicial prior to extraction (g)}} \text{ x } 100\%$$ (Wijaya *et al*., 2018)

c) Phytochemical screening

Phytochemical screening was conducted to identify key bioactive compounds in the plant extract, and it allowed for a preliminary assessment of the extract's bioactive components. Phytochemical screening includes the identification of alkaloids using Dragendorff reagent, flavonoids using $AlCl_3$, foam tests for saponin, tannins with $FeCl_3$ reagent, quinones using H_2SO_4, and terpenoid/steroid compounds using Liebermann Burchard method (Yuda *et al*., 2017).

d) Profile of thin-layer chromatography

One hundred milligrams of thick ethanol extracts from *L. sarmentosa* root were measured. The extract was solubilized in 10 mL of ethanol and subsequently filtered using filter paper. The *L. sarmentosa* roots extract solution was spotted onto a GF254 silica gel plate and further eluted with polar ethyl acetate eluent:n-hexane (7:3) and a non-polar ethyl acetate eluent:n-hexane (3:7) solvent system. Next, the eluted plates were examined under UV light at 254 nm to reveal the stains.

2.3.2. Non-specific parameters

a) Determination of the water content

The water content was determined using the toluene distillation technique. Toluene is first saturated with water, agitated until homogeneous, and then allowed to settle until it separates into two layers (the toluene phase and the water phase). The toluene layer is used as the solvent to determine the water content. After weighing 1 g of *L. sarmentosa* root extract, 40 mL of saturated toluene was added to a round-bottom flask. Following 100 minutes of boiling distillation, the mixture was cooled to room temperature. The observed volume of water was then converted into a percentage (Depkes RI, 2008). The maximum limit of water content is ≤ 10% (BPOM RI, 2019).

b) Determination of the overall ash content

The amount of 2 g of extract, which had been meticulously weighed and crushed, was placed in a heated silicate crucible and leveled. Then, it was cooled after slowly heating the charcoal until it was gone prior to weighing it. When this technique fails to eliminate the charcoal, heated water is introduced, and the solution is filtered via ash-free filter paper. Filter paper and the residual paper are both heated in the identical crucible. The filtrate was placed to the crucible, then it was evaporated, heated, and weighed to a fixed weight. The ash content is calculated through the air-dried material (Depkes RI, 2000).

c) Determination of acid insoluble ash

The ashes acquired during ash content analysis were subjected to boiling for 5 minutes in 25 mL of diluted hydrochloric acid. The insoluble fraction of the acid was gathered, and the ash was filtered using ash-free filter paper and subsequently washed with hot water. The residue and filter paper were burnt until a constant weight was attained. The acid-insoluble ash content was determined using the air-dried sample (Depkes RI, 2000).

d) Determination of heavy metals contamination

Heavy metal contamination (Pb, Cd, and Hg) can be analyzed using the atomic absorption spectrophotometry (AAS) method. A total of 1 g of extract was added to 10 mL of concentrated HNO_3. The mixture was then heated until it becomes thick or dry. Once the extract was thick and cooled, 10 mL of distilled water and 5 mL of perchloric acid were added. The mixture was re-heated again until thick and then filtered into a 50 mL volumetric flask. Distilled water is added up to 50 mL, and the sample is measured using the AAS method (Depkes RI, 2000). Lead (Pb) contamination is limited to ≤ 10 mg/kg or mg/L, cadmium (Cd) to ≤ 0.3 mg/kg or mg/L, and mercury (Hg) to ≤ 0.5 mg/kg or mg/L, as per BPOM RI regulation (2019).

2.3.3. Total flavonoid determination

Quantitative analysis was performed using UV-Vis spectrophotometry to determine the total flavonoid content of the 70% ethanol extract of *L. sarmentosa* root. The total flavonoid content was determined using a colorimetric method with $AlCl_3$ as a reagent and quercetin as the standard.

3. Result and discussion

The identification of the *L. sarmentosa* sample confirmed that the saluang belum plant from Timpah Village, Kapuas Regency, Central Kalimantan, belongs to the species *Luvunga sarmentosa* (Blume) Kurz and is classified under the Rutaceae family, as stated in certificate 065/LB.LABDASAR/II/2023. Two kilograms of *L. sarmentosa* roots, from mature plants at least 2 meters tall, were collected, washed, sliced, and dried at 55°C for eight hours. The dried roots were then ground into powder (simplicia) and stored in an airtight container, protected from direct sunlight. From the initial collection, 1201.5 grams of simplisia powder were obtained, equating to a yield of 60.075%. The simplicia powder was further subjected to maceration using 96% ethanol. A total of 250 grams of the powdered roots were processed, resulting in 21.44 grams of extract, which represents an extraction efficiency of 8.57%.

3.1. Specific parameters

3.1.1. Characteristics of the extract

Extract description (**Table 1**) was conducted by administering a questionnaire regarding the shape, color, odor, and flavor of the *L. sarmentosa* root extract sample to 5 panelists. The viscous extract was procured through the process of evaporation, employing a rotary evaporator in conjunction with a water bath maintained at a temperature of 50°C. The hue of the resultant extract manifests as a yellowish-brown shade. The alteration in colour can be attributed to the oxidation reactions prompted by the thermal effects of the filtrate's evaporation into extracts (Ramayani *et al*., 2021). The scent and taste generated by the thick extract of *L. sarmentosa* roots can be ascribed to the secondary metabolites contained within, which include bitter-tasting substances like flavonoids and alkaloids (Hasby *et al*., 2019).

Table 1. Results of the description of *L. sarmentosa* root extract

Figure	Shape	Color	Odor	Flavor
	viscous	yellowish brown	strong, distinct	bitter

3.1.2. Phytochemical screening

Table 2. Phytochemical result of *L. sarmentosa* roots extract

No	Phytochemical compounds	Test results	Conclusion
1.	Alkaloid	Sediment orange and yellow	Positive
2.	Flavonoid	Color of solution red yellowish	Positive
3.	Saponins	Not formed foam	Negative
4.	Tannin	Green-black color of the solution	Positive
5.	Quinone	No color solution changed	Negative
6.	Steroid	Formed ring blue greenish	Positive
7.	Terpenoid	Formed ring brownish	Positive

The phytochemical screening test (**Table 2**) shows that viscous extracts of *L. sarmentosa* roots contain alkaloids, flavonoids, tannins, terpenoids, and steroids. The identification of steroid compounds was carried out using the thin layer chromatography (TLC) method. The Liebermann-Burchard reagent sprayed on a plate coated with *L. sarmentosa* root extract, followed by heating in an oven at 105°C for 5 minutes. The TLC results showed a green stain, indicating the positive presence of steroids. Positive steroid reactions are confirmed by the appearance of blue-green stains (Yuda *et al*., 2017). According to Anggriani's research (2018), the roots of *L. sarmentosa* also tested positive for steroids and flavonoids using TLC. Additionally, another study identified alkaloids, terpenoids, flavonoids, tannins, and steroids in the roots of *L. sarmentosa* using LC-MS (Syarpin *et al*., 2023). Qualitative testing is based on observing color changes that occur after the sample is treated with specific reagents. A negative test result, indicating the absence of a compound, may be due to the compound being present in very low concentrations in the extract.

3.1.3. Thin layer chromatography (TLC) profile

The TLC profile of *L. sarmentosa* root extracts (**Figure 1**), utilizing a nonpolar mobile phase of ethyl acetate:n-hexane (3:7) and examined under UV illumination at 254 nm, exhibited four spots with Rf values between 0.56 and 0.88 (**Table 3**). The TLC profile utilizing a polar mobile phase of ethyl acetate:n-hexane (7:3) under identical UV light conditions exhibited six spots with Rf values between 0.58 and 1.08. The spots and Rf values were examined under a 254 nm UV light, generating a luminous background, while the sample manifested as dark spots. The identification extract utilizing TLC seeks to observe the separation of samples through characteristic chromatogram patterns, which arise from polarity discrepancies between the sample and the solvent (eluent), thereby providing insights into the initial chemical composition based on the chromatogram pattern (Depkes RI, 2000). The Rf value was ascertained for the purpose of compound identification. The Rf value is the ratio of the distance travelled by the eluent to the phase movement on the TLC plate, serving as a comparative metric among samples (Sopiah *et al*. 2019).

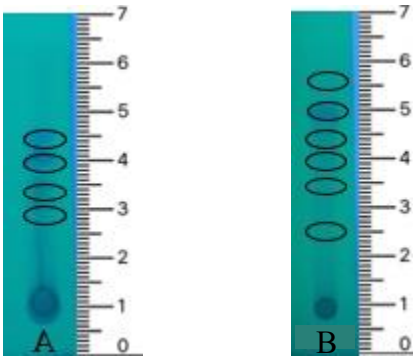

Figure 1. TLC results under $UV_{254\ nm}$ with (**A**) ethyl acetate:n-hexane (3:7) v/v and (**B**) ethyl acetate:n-hexane (7:3) v/v as mobile phase.

Table 3. Rf value of *L. sarmentosa* root extract using n-hexane:ethyl acetate mobile phase under 254 nm UV light

Non polar mobile phase/ ethyl acetate:n-hexane (3:7)	Polar mobile phase/ ethyl acetate:n-hexane (7:3)
$Rf_1 = \frac{2,8}{5} = 0.56$	$Rf_1 = \frac{2,9}{5} = 0.58$
$Rf_2 = \frac{3,3}{5} = 0.66$	$Rf_2 = \frac{3,6}{5} = 0.72$
$Rf_3 = \frac{3,9}{5} = 0.78$	$Rf_3 = \frac{4,0}{5} = 0.8$
$Rf_4 = \frac{4,4}{5} = 0.88$	$Rf_4 = \frac{4,5}{5} = 0.9$
	$Rf_5 = \frac{4,8}{5} = 0.96$
	$Rf_6 = \frac{5,4}{5} = 1.08$

3.2. Nonspecific parameters

3.2.1. Determination of the water content

This study used the toluene distillation method, which fundamentally utilizes toluene-saturated water, to ascertain the water content. Toluene is saturated, thereafter mixed with water, agitated until homogeneous, and allowed to stand until two distinct layers are created (aqueous and toluene phase). Layer toluene is used as a solvent in the determination of water content. Weigh the extract root *L. sarmentosa* as much as 1 g, then enter it into a round-bottom flask, and 40 mL of toluene was added to saturation. Distil for 100 minutes until boiling, then cool down until reaching room temperature. The volume of water obtained was observed and calculated in percentage form (Depkes RI, 2008). The result of determining the water content of ethanol extracts of *L. sarmentosa* roots is 2.43 ± 0.15%. The stipulations outlined in this requirement have been satisfied, as the water content is below 10%. Water content determination is conducted to assess the residual water remaining after the drying or concentration process, with the aim of establishing a permissible range or maximum value for the extract's moisture content. This determination is crucial because high water levels can provide a favourable environment for bacteria and fungi, which may degrade the

extract's constituent compounds (Wahyuni & Anggelina, 2021). Additionally, water content is linked to the extract's purity. Excessively high-water content (>10%) can promote microbial growth, which in turn reduces the stability of the extract (Utami *et al.*, 2017).

3.2.2. Determination of total ash content and acid insoluble ash content

Determination of ash content has the principle of oxidizing inorganic substances at high temperatures (600°C) and then weighing the substances left behind during the ashing process; the higher the ash content, the more mineral content in the extract (Maulana, 2016). The total ash content of the ethanol extract from *L. sarmentosa* roots was determined to be 1.50% ± 0, meeting the requirement of less than 1.9% (Kemenkes RI, 2017). This result satisfies the specified guidelines. Higher total ash content indicates a greater presence of minerals in the material or sample, which may include calcium, phosphorus, magnesium, sodium, chloride, or heavy metals such as mercury, lead, copper, and others (Utami *et al.*, 2017). Additionally, the acid-insoluble ash content of the *L. sarmentosa* root extract was found to be 1.00% ± 0.86, also meeting the requirement of less than 1.5% (Kemenkes RI, 2017). This result complies with the outlined stipulations. Acid-insoluble ash content serves as an indicator of metal or mineral contamination that is insoluble in acid. Elevated acid-insoluble ash content suggests the presence of silicates from sand or soil, as well as metals such as silver, lead, and mercury (Utami *et al.*, 2017).

3.2.3. Determination of heavy metal contamination (Pb, Cd, and Hg)

Natural components in the soil, known as heavy metals, are incapable of degradation or destruction. These compounds can infiltrate the human body through food, drinking water, and air (Hardani *et al*., 2022). Heavy metal contamination is a quality requirement that needs to be considered in the manufacture of traditional medicines (Susanti *et al*., 2023). The test results for heavy metal contamination in *L. sarmentosa* root extract showed a lead (Pb) content of <0.001 mg/kg. The permissible limit for Pb contamination is ≤10 mg/kg or mg/L, so the results comply with the established requirements. However, the cadmium (Cd) contamination level in the root extract was found to be 0.446 mg/kg, exceeding the permissible limit of ≤0.3 mg/kg or mg/L, meaning the result does not meet the requirements. Additionally, the mercury (Hg) content was 2.077 mg/kg, also exceeding the allowed limit of ≤0.5 mg/kg or mg/L, indicating the result does not comply with the regulations (BPOM RI, 2019). Based on this data, *L. sarmentosa* root extract is contaminated with Cd and Hg heavy metals. This contamination may be attributed to natural processes or pollution from human activities. Cd contamination can originate from the combustion of domestic waste, coal, or other fossil fuels (Syamsuddin & Rasjid, 2022). Mercury is a naturally occurring element found in the environment in metallic form, as mercury salts, and as organic mercury. It enters the environment

through the natural breakdown of minerals in rocks and soil due to exposure to wind and water. However, human activities such as fossil fuel combustion, mining, smelting, and solid waste incineration cause the majority of Hg pollution (Irianti *et al.*, 2021). The solution to this problem is to cultivate *L. sarmentosa* in protected environments that are shielded from heavy metal pollution. The procedure includes maintaining controlled soil conditions and pH levels to minimize the heavy metal content in saluang belum, ensuring it is safe for consumption or for processing into herbal products.

3.3. Total flavonoid determination

The determination of total flavonoid content in *L. sarmentosa* used an extract with 70% ethanol as the solvent. According to Riwanti *et al*. (2020), flavonoid compounds, which are polar in nature, tend to dissolve more effectively in 70% ethanol, resulting in a higher flavonoid content compared to 96% ethanol.

The standard curve determination aims to calculate sample concentration using a regression equation based on the known absorbance of a standard. When concentration and absorbance are directly proportional, the r value indicates a linear curve (Suharyanto & Hayati, 2021). A quercetin standard solution with concentrations of 20, 40, 60, 80, and 100 ppm was used. Absorbance was measured with a UV-Vis spectrophotometer at 433.40 nm after 30 minutes of incubation. This is because the reaction between quercetin and $AlCl_3$ produces a yellow solution that absorbs light in the blue wavelength range of 400-435 nm (Kent, 2012). The standard curve for quercetin was replicated three times for each concentration, resulting regression equation y = 0.0066x – 0.0183 with r value of 0.9979.

Table 4. Results of the total flavonoid content analysis from the ethanol 70% extract of *L. sarmentosa* root

Replication	Abs. sample	Total flavonoid content (%w/w QE)	$\bar{x}$ total flavonoid content (%w/w QE) ± SD	RSD (%)
1	0.4381	6.9152		
2	0.4375	6.9061	6.9147 ± 0.0083	0.12
3	0.4386	6.9227		

The total flavonoid content from the 70% ethanol extract of *L. sarmentosa* root, based on three replications (**Table 4**), averaged 6.9147 ± 0.0083 %w/w QE, which is slightly higher than the findings of a previous study by Wathan *et al*. (2023) using a 96% ethanol extract, with a content of 6.56 ± 0.006 %w/w extract. The RSD values of the three replications met the criteria for satisfactory RSD (<2%) due to their low variability, indicating consistent and reliable results.

4. Conclusion

All specific and non-specific parameters of the extract meet the standards, except for the heavy metal pollutants Hg and Cd, which remain under the threshold levels established by BPOM. Consequently, we must conduct an analysis to ascertain the concentration of metallic weight present in the root of *L. Sarmentosa*. The total flavonoid content was determined to be 6.9147 ± 0.0083% w/w QE. This research should be extended to investigate additional characteristics not addressed in this work, including microbial contamination, arsenic as a heavy metal contaminant, and the identification of specific chemicals.

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References

Anggriani, M. F. (2018). Identifikasi dan Potensi Senyawa Metabolit Sekunder Pada Akar Tumbuhan Saluang Belum (*Lavanga sarmentosa* (Blume) Kurz) Sebagai Afrodisiak. *Jurnal Ilmiah Kanderang Tingang*, *9*(1), 97–102.

Aulani, F. N. (2018). Cara BPOM Memastikan Keamanan Obat Tradisional di Masyarakat. *Majalah Farmasetika*, *3*(2), 30–32. https://doi.org/10.24198/farmasetika.v3i2.21620

BPOM RI. (2019). *Peraturan Badan Pengawas Obat dan Makanan Nomor 32 Tahun 2019 Tentang Persyaratan Keamanan dan Mutu Obat Tradisional*. Jakarta: Badan Pengawas Obat dan Makanan Republik Indonesia.

Depkes RI. (2000). *Parameter Standar Umum Ekstrak Tanaman Obat*. Jakarta: Departemen Kesehatan RI

Depkes RI. (2008). *Farmakope Herbal Indonesia Edisi I*. Jakarta: Departemen Kesehatan Republik Indonesia.

Hardani, P. T., Pramushinta, I. A. K., & Purbosari, I. (2022). Penyuluhan Pemanfaatan Belimbing Wuluh (*Oreochromis Mossambicus*) dan Asam Sitrat Untuk Mengurangi Cemaran Logam Berat Pada Ikan. *Pancasona*, *1*(1), 23–28. https://doi.org/10.36456/pancasona.v1i1.6153

Hasby, Nurhafidhah, Mauliza, Wati, Z., & Adelina, R. (2019). *Pemanfaatan Metabolit Sekunder dalam Berbagai Bidang*. Klaten: Lakeisha.

Irianti, T. T., Kuswandi, Nuranto, S., & Purwanto. (2021). *Antioksidan dan Kesehatan*. Yogyakarta: Gadjah Mada University Press.

Kemenkes RI. (2017). *Farmakope Herbal Indonesia Edisi II*. Jakarta: Kementerian Kesehatan Republik Indonesia.

Kent, J. A. (2012). *Handbook of Industrial Chemistry and Biotechnology*. New York: Springer.

Marjoni, R. (2022). *Monografi: Potensi Antioksidan Ekstrak Kulit Buah Sukun (Artocarpus altilis)*. Sleman: Resitasi Pustaka.

Marpaung, M. P., & Septiyani, A. (2020). Penentuan Parameter Spesifik dan Nonspesifik Ekstrak Kental Etanol Batang Akar Kuning (*Fibraurea chloroleuca* Miers). *Journal of Pharmacopolium*, *3*(2), 58–67. https://doi.org/10.36465/jop.v3i2.622

Maulana, A. (2016). *Analisis Parameter Mutu dan Kadar Flavonoid Pada Produk Teh Hitam Celup*. Tugas Akhir. Program Studi Teknologi Pangan, Fakultas Teknik, Universitas Pasundan Bandung.

Musfirah, Y., Bachri, M. S., & Nurani, L. H. (2016). Efek Ekstrak Etanol 70% Akar Saluang Balum (*Lavanga sarmentosa*, *Blume kurz*) Terhadap Spermatogenesis dan Gambaran Histopatologik Testis Mencit. *Jurnal Pharmascience*, *3*(2), 131–141.

Najib, A., Malik, A., Ahmad, A. R., Handayani, V., Syarif, R. A., & Waris, R. (2017). Standarisasi Ekstrak Air Daun Jati Belanda Dan Teh Hijau. *Jurnal Fitofarmaka Indonesia*, *4*(2), 241–245. https://doi.org/10.33096/jffi.v4i2.268

Ramayani, S. L., Permatasari, E. A., Novitasari, I., & Maryana. (2021). Pengaruh Metode Ekstraksi Terhadap Kadar Total Fenolik, Kadar Total Flavonoid dan Aktivitas Antioksidan Ekstrak Daun Mengkudu (*Morinda citrifolia* L.). *Jurnal Ilmu Farmasi Dan Farmasi Klinik*, *18*(1), 40–46. https://doi.org/10.37013/jf.v10i1.115

Riwanti, P., Izazih, F., & Amaliyah. (2020). Pengaruh Perbedaan Konsentrasi Etanol pada Kadar Flavonoid Total Ekstrak Etanol 50,70 dan 96% Sargassum polycystum dari Madura. *Journal of Pharmaceutical Care Anwar Medika Artikel*, *2*(2), 82–95.

Sopiah, B., Muliasari, H., & Yuanita, E. (2019). Skrining Fitokimia Dan potensi Aktivitas Antioksidan Ekstrak Etanol Daun Kastuba (*Euphorbia pulcherrima* Willd.). *Jurnal Ilmu Kefarmasian Indonesia*, *17*(1), 27–33.

Suharyanto, S., & Hayati, T. N. (2021). Penetapan Kadar Flavonoid Total Ekstrak Buah Gambas (*Luffa acutangula*(L.) Roxb.) dengan Metode Spektrofotometri UV-Vis. *Pharmacon: Jurnal Farmasi Indonesia*, *18*(1), 82–88. https://doi.org/10.23917/pharmacon.v18i01.10916

Susanti, M. M., Ariyanti, D. D., Ardianti, S., & Mahanani, W. F. C. (2023). Pengaruh Tempat Tumbuh Terhadap Kadar Logam Berat Timbal (Pb), Cadmium (Cd) dan Tembaga (Cu) Ekstrak Rimpang Jahe Emprit (*Zingiber officinale Var. amarum*) Dengan Metode Spektrofotometri Serapan Atom. *Jurnal Inovasi Teknik Kimia*, *8*(1), 34–39. https://doi.org/10.31942/inteka.v18i1.8093

Syamsuddin, S., & Rasjid, A. (2022). *Penggunaan Ampas Kopi dalam Menurunkan Parameter Kimia (Cd) pada Air Lindi.* Yogyakarta: Nas Media Indonesia.

Syarpin, Permatasari, S., & Pujianto, D. A. (2023). Analysis of Phytochemical Constituents and Antioxidant Activity From The Fractions of Luvunga sarmentosa Root Extract Using LCMS/MS. *Biodiversitas*, *24*(2), 733–740. https://doi.org/10.13057/biodiv/d240208

Utami, P. Y., Umar, H. abdul, Syahruni, R., & Kadullah, I. (2017). Standardisasi Simplisia dan Ekstrak Etanol Daun Leilem (*Clerodendrum minahassae Teisjm. & Binn.*) Reny Syahruni Sekolah Tinggi Ilmu Farmasi Makassar. *Journal of Pharmaceutical and Medicinal Sciences*, *2*(1), 32–39. https://www.researchgate.net/publication/350241362

Wahyuni, Y. S., & Anggelina, S. (2021). Penetapan Kadar Senyawa Terlarut Dalam Pelarut Etanol dan Kadar Air Ekstrak Daun Jambu Mete (*Anacardium occidentale* L.) Sebagai Parameter Spesifik dan Non Spesifik. *Jurnal Kesehatan Yamasi Makassar*, *5*(1), 105–111.

Wardah, & Sundari, S. (2019). Ethnobotany study of Dayak society medicinal plants utilization in Uut Murung District, Murung Raya Regency, Central Kalimantan. *IOP Conference Series: Earth and Environmental Science*, *298*(1), 1–12. https://doi.org/10.1088/1755-1315/298/1/012005

Wathan, N., & Rizki, M. (2020). Penentuan Aktivitas Antioksidan Ekstrak Etil Asetat dan Ekstrak Metanol Akar Saluang Belum (*Luvunga sarmentosa*). *Prosiding Seminar Nasional Lingkungan Lahan Basah*, 126–128.

Wathan, N., Rizki, M. I., Khairunnisa, A., & Simamora, H. (2023). Total Flavonoids Determination and Antioxidant Activity of Ethyl Acetate, Ethanol, and Methanol Extracts from Seluang Belum Root (*Luvunga sarmentosa (Blume) Kurz.*). *Berkala Kedokteran*, *19*(1), 101–106. https://doi.org/10.20527/jbk.v19i1.15733

Wijaya, H., Novitasari, & Jubaidah, S. (2018). Perbandingan Metode Ekstraksi Terhadap Rendemen Ekstrak Daun Rambai Laut (*Sonneratia caseolaris L. Engl*). *Jurnal Ilmiah Manuntung*, *4*, 79 – 83.

Yuda, P. E. S. K., Cahyaningsih, E., & Winariyanthi, N. P. Y. (2017). Skrining Fitokimia dan Analisis Kromatografi Lapis Tipis Ekstrak Tanaman Patikan Kebo (*Euphorbia hirta* L.). *Jurnal Ilmiah*

Medicamento, *3*(2), 61–70. https://doi.org/10.36733/medicamento.v3i2.891

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Update on pharmacist role in sterile compounding in hospital

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Abstract

Background: Sterile compounding, a critical aspect of pharmaceutical practice, involves the preparation of customized medications in a sterile environment. Pharmacists play a pivotal role in ensuring the safety and efficacy of compounded sterile products (CSPs), making their involvement integral to the healthcare system.

Objective: This article aims to describe the role of pharmacists in sterile compounding based on literature.

Method: Articles from Medline/PubMed, guidelines, reports, and databases related to sterile compounding have been searched and compiled. This review collected the qualitative data and identified the critical point for continuous quality improvement initiatives in sterile compounding.

Results: This comprehensive narrative review explores the multifaceted responsibilities of pharmacists in sterile compounding, drawing insights from various studies and databases. However, the study related to the pharmacist's role in sterile compounding practices is very limited. Recent articles that mention the pharmacist's role in sterile compounding commonly come from the guidelines or government documents. In general, CSP needs a commitment to comply with regulatory standards for achieving patient safety using the integration of technology and healthcare collaboration. Continuous professional development is a crucial contributor to keeping the quality of compounded sterile products.

Conclusion: The findings from various journals underscore the complexity of their responsibilities and emphasize the need for a holistic approach to ensure the integrity and safety of compounded sterile products in diverse healthcare settings.

Keywords: Pharmacist's role, patient safety, sterile compounding

1. Introduction

Sterile compounding is a specialized area within pharmaceutical practice that involves the preparation of customized medications in a sterile or aseptic environment to achieve free from any contaminations (USP, 2023). This process is crucial for patients who require medications that are not commercially available or need specific formulations tailored to the individual needs, such as intravenous admixture and parenteral nutrition (Hanifah *et al*., 2021). Compounded sterile products (CSPs) are typically injectable medications, intravenous solutions, or other sterile dosage forms which are under pharmacist's responsibility that should be (USP, 2023).

Pharmacists play a pivotal role in ensuring the safety and efficacy of medications, making their involvement integral to the healthcare system (Saseen *et al*., 2017). The pharmacist is the bridge between a physician/surgeon and patients who counsel and advise the

patient to maximize the desired effect of the drugs and minimize the untoward/adverse effects of the drug (Minor *et al*., 2019). In many types of health facilities, a pharmacist or drug expert is an individual who is engaged in designing, creating or manufacturing of a drug product, dispensing of a drug, managing, and planning of a pharmaceutical care plan also making sure the administration (Abdelaziz *et al*., 2016). They are experts in the activity and use of drugs, including their chemistry, pharmacology, and the formulation of medicines. They are the health care professionals who have the responsibility to give essential consideration to the patients and giving protection and providing safe and effective use of medicines (Islam *et al*., 2015). Pharmaceutical care is a comprehensive and patient-cantered approach in pharmacy practice that emphasizes collaboration among healthcare providers to optimize drug therapy outcomes (Tran *et al*., 2017). The concept has evolved since its initial definition by Hepler and Strand in 1990, which stated that pharmaceutical care is "the responsible provision of drug therapy for the purpose of achieving definite outcomes that improve a patient's quality of life" (Helgesen *et al*., 2024). This definition highlights the pharmacist's role in ensuring safe and effective medication use while being accountable for patient outcomes. Therefore, pharmaceutical care represents a critical component of modern healthcare, where pharmacists actively engage in the therapeutic process alongside other healthcare providers to ensure safe, effective, and personalized medication management for patients.

However, sterile compounding is currently a new role in hospital compared to the non-sterile preparation. Health care facilities have applied various sterile medication to be prepared in hospital. Furthermore, the facilities also have a variety in function and complexity which determine different role of pharmacist. This review wants to know the pharmacist' roles and how they work in sterile dispensing unit based on regulation also the real work in hospital. This comprehensive narrative review explores the multifaceted responsibilities of pharmacists in sterile compounding, drawing insights from various journals and studies.

2. Method

The literature search was conducted using databases such as Google Scholar, PubMed, and Elsevier, which are published during 2016 to 2023. The keywords applied during the topic search process from relevant articles were "pharmacist", "role", and "sterile compounding". The studies were identified then categorized to answer the topics of regulation and the reason of sterile preparation also the works of pharmacist and the supporting factor to improve. Then,

the results of manual review were categorized as regulatory (13 studies) also pharmacist's role (20 studies). Supporting factors that were identified are including the technological advancement and interprofessional collaboration (33 studies).

3. Result and discussion

Pharmacists have experienced a very rapid shift in roles since the 1960s (Hanifah *et al.*, 2022). In terms of compounding, the role of pharmacists is very critical because there is no other profession that can competently replace it (Myers, 2013). Sterile compounding activities are clearly stated as part of pharmaceutical work in hospitals (Kemenkes RI, 2016). Pharmacists play a role starting from ensuring the quality of preparations, prescription screening, aseptic preparation, to ensuring the final quality of the product before being given to medical personnel and patients. In carrying out this role, pharmacists need to continue to update themselves with developments in knowledge, regulatory changes, and technological developments, as well as building a collaborative work culture with other health workers.

Based on the search strategy, research or writings on the role of pharmacists in sterile compounding are very limited. In fact, no articles can be obtained from a Google Scholar search using the keyword "pharmacist role in sterile compounding". New search process can be obtained with the keywords sterile compounding in hospital pharmacy. Writings that describe the role of pharmacists in sterile compounding are generally in popular or opinion articles. Meanwhile, writings on sterile compounding are mostly obtained from guidelines or articles which linked with technological developments, the importance of collaboration and continuous improvement.

3.1. Regulatory compliance and standards

One of the primary responsibilities of pharmacists in sterile compounding is ensuring strict adherence to regulatory standards. According to Smith *et al.* (2019), pharmacists must navigate and implement guidelines established by organizations such as the United States Pharmacopeia (USP), European Association Hospital Pharmacy (EAHP) and Institute for Safe Medication Practice (ISMP). Currently, USP 797 is a gold standard which is referred to by many countries for sterile compounding. As seen on **Table 1**, EAHP guide as how pharmacist engaged in sterile compounding, but USP 797 guides the detail standard of personnel, room and facilities, the procedure also the category of compounding sterile preparation which will be

basis of beyond used date (USP, 2023). Currently, the standard of BUD (beyond use date) is mostly referred to as USP. Compliance with these standards involves meticulous attention to detail, rigorous aseptic techniques, and continuous monitoring of environmental conditions to maintain the integrity of CSPs. Other guidelines like EAHP and ISMP address the general principles of safety and supporting system including the staff. From three guidelines emphasize the safety which are mostly influenced with the staffs mainly pharmacist (EAHP, 2023).

Table 1. The scope of standards for guiding sterile preparation

United State Pharmacopeia (USP) 797 last updated 2023	**European Association Hospital Pharmacy (EAHP) 2023**	**ISMP guidelines for sterile compounding and the safe use of sterile compounding technology last updated 2016**
Personal	The importance of compounding for addressing patient needs	Essential technology attributes
Facility	Engaging hospital pharmacists in the provision of personalized medication	Safe pharmacy processes
Category of CSP	Adjusting education and training to the increased need for personalised care	Safety gaps in three areas: automated compounding devices, IV workflow management systems, and IV robots.
Beyond use date	The involvement of hospital pharmacies in reconstitution practices	
Preparing, repackaging, labelling	The role of hospital pharmacists in the preparation and use of ATPMs	

3.2. Pharmacist' role in sterile compounding

To ensure the safety and efficacy of compounded sterile medication, pharmacist have to follow the guidance (Lam & Sokn, 2019). Pharmacists must adhere to strict regulations and guidelines set forth by regulatory bodies and other international standards. Compliance with these standards is critical to maintaining the quality and safety of compounded sterile products. Pharmacists play a key role in identifying and mitigating potential risks associated with sterile compounding. This includes assessing the risk of microbial contamination, human errors, and other factors that could compromise the integrity of compounded products (Whyte, 2004).

Pharmaceutical compounding is a critical aspect of pharmacy practice, involving the preparation of customized medications that requires a blend of scientific knowledge, technical skill, and regulatory compliance. Compounding pharmacists ensure that medications are tailored precisely to meet individual patient needs while adhering to stringent quality standards. This process typically includes combining, mixing, or altering drug ingredients to create formulations that are not commercially available or to modify existing medications to better suit patient requirements (Croft *et al*., 2018).

During preparation, all personnel must follow established protocols for aseptic technique, which involves maintaining a sterile environment to prevent contamination during the compounding process. Pharmacists are involved in quality assurance measures to guarantee the compatibility, stability and sterility of compounded products (Hanifah *et al*., 2022). This may include regular testing of air quality, equipment, and finished products to detect and prevent microbial contamination (Qadus *et al*., 2022). They must adhere to strict aseptic techniques to prevent microbial contamination during the compounding process (Ayalew *et al*., 2019). This includes maintaining a sterile environment, using appropriate personal protective equipment, and employing proper hand hygiene practices. Recent literature highlights the pharmacist's pivotal role in ensuring the quality and safety of compounded sterile preparations (CSPs). A study by Truong *et al.* (2020) emphasizes the need for continuous training and education for pharmacists engaged in sterile compounding to enhance their competencies and minimize the risk of errors. Furthermore, the pharmacist's role extends to regular monitoring and testing of compounded products. Frequent quality control assessments, including microbial testing and potency checks, are essential to guarantee the integrity of sterile medications.

Quality control in hospital compounding pharmacies is essential to ensure the safety and effectiveness of compounded medications (**Figure 1**). By employing thorough visual inspection methods and adhering to comprehensive QA protocols, pharmacists can ensure that compounded medications are safe and effective for patient use post-preparation. Any particles, discoloration, gas formation, or other visible changes indicate disqualification. Pharmacists should reject this product and evaluate to improve the quality.

To achieve patient safety in sterile compounding, pharmacists also significantly contribute to keep each step and aspect (Dalton & Byrne, 2017). According to a comprehensive review by Brown and White (2021), pharmacists play a crucial role in the entire process, from prescription assessment to the final administration of CSPs. They are responsible for conducting risk assessments, identifying potential sources of contamination, and implementing strategies to mitigate these risks. Ultimately, pharmacists are advocates for patient safety. They ensure that compounded medications meet the highest standards of quality and are free from contaminants, helping to prevent adverse effects and ensure positive patient outcomes (Aprilliano *et al*., 2023).

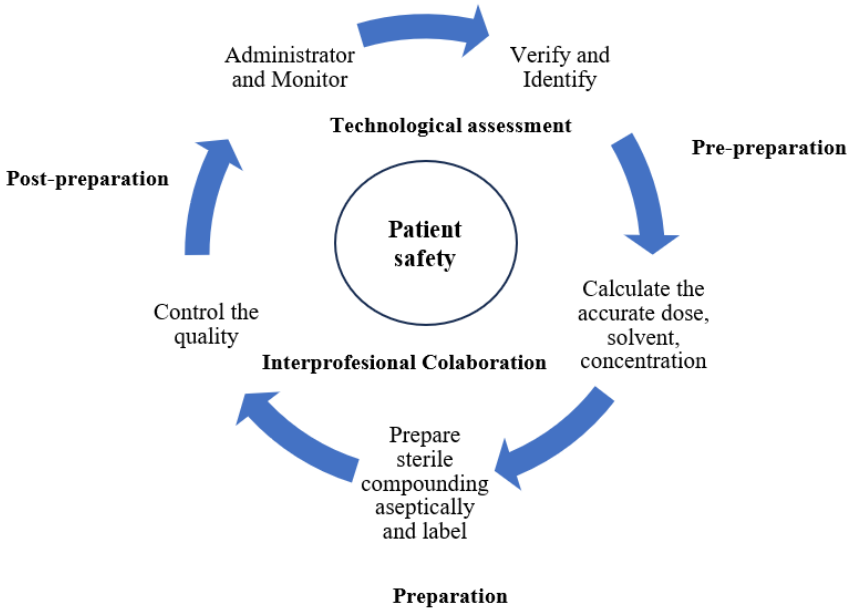

Figure 1. Pharmacist's role in sterile compounding

Moreover, pharmacists collaborate with other healthcare professionals to ensure that compounded products meet the unique needs of individual patients while aligning with therapeutic goals (Brown & White, 2021). This collaborative approach enhances patient safety by incorporating diverse perspectives and expertise, emphasizing the pharmacist's role as an essential member of the healthcare team. Pharmacists also collaborate with other healthcare professionals to ensure seamless integration of compounded medications into patient care plans (Alhamoudi & Alnattah, 2018).

To improve and meet high qualification as above, pharmacists are tasked with training and educating compounding personnel, including pharmacy technicians, on proper sterile compounding techniques. Continuous education is crucial to staying updated on best practices and emerging technologies in the field (Mill *et al*., 2021). Pharmacists are also responsible for maintaining thorough documentation of the compounding process, including ingredient measurements, environmental monitoring data, and quality control results. Accurate record-keeping is essential for traceability and accountability (Lee *et al*., 2023). Furthermore, Johnson and Davis (2020) emphasize the pharmacist's role in minimizing the risk of contamination during compounding processes. Pharmacists are instrumental in selecting sterile ingredients, ensuring proper storage, and employing appropriate administration techniques. This commitment to compliance not only meets regulatory requirements but also safeguards patients from potential harm and highlights the pharmacist's dedication to patient safety.

3.3. Technological advancements in sterile compounding

The landscape of sterile compounding has been significantly influenced by technological advancements. Lee and Chen (2018) highlight the use of automated compounding systems and robotics in their review, emphasizing how pharmacists embrace these innovations to improve precision and reduce the likelihood of errors. Automation not only enhances the efficiency of the compounding process but also contributes to increased accuracy in dosage calculations and product preparation (Fan *et al*., 2022). Furthermore, Fan found that technology-assisted workflow system (TAWS) in sterile compounding safety able to increase the ability to check, trace, and detect the errors also increase efficiency by reducing processing time (Fan *et al*., 2022). Automation of compounding tools proved the benefits of sterile preparation by reducing errors and processing time. Beyond the instrument for preparing, technological advancement for sterilization, sterilization control, and supporting tools like laminar air flow, personal protective equipment (PPE) is also significant to achieve patient safety.

To adapt with the advancement of technology, pharmacists, through ongoing training and education, integrate technology seamlessly into their compounding practices. This adaptability reflects their commitment to staying abreast of new developments, ensuring that patients receive CSPs prepared with the latest technological advancements, ultimately contributing to improved patient outcomes (Jean *et al*., 2020).

3.4. Interdisciplinary collaboration

Effective communication and collaboration among healthcare professionals are essential for successful sterile compounding. Brown and White (2021) underscore the pharmacist's role in interdisciplinary teamwork, emphasizing the importance of open communication with physicians, nurses, and other healthcare providers. This collaboration ensures that the entire healthcare team is aligned in providing safe and effective CSPs to patients. This study emphasizes the pharmacist's role within an interdisciplinary healthcare team. Interdisciplinary teamwork involves collaboration between professionals from different healthcare disciplines, such as pharmacists, physicians, nurses, and other specialists (Agomo *et al*., 2016). Effective communication is crucial for the success of sterile compounding. Open lines of communication between pharmacists and other healthcare providers ensure that everyone

involved in the patient's care is well-informed about the compounded sterile products (CSPs) being used (Mustikawati *et al*., 2023).

In their study, Garcia and Patel (2019) further elaborate on the necessity of interdisciplinary collaboration, emphasizing how pharmacists bring their unique expertise to the table. This collaboration extends beyond the compounding process, involving consultations with other healthcare professionals to tailor CSPs according to specific patient needs, medical histories, and treatment plans. This study highlights that pharmacists bring unique expertise to the interdisciplinary team. Pharmacists play a pivotal role in sterile compounding beyond the actual preparation of medications (Abdulghani *et al*., 2017). Pharmacists use their pharmaceutical knowledge to consult with other healthcare professionals, including physicians and nurses, to customize compounded medications according to specific patient needs. This involves considering individual patient characteristics, medical histories, and treatment plans (Kim *et al*., 2023).

The collaboration between healthcare professionals extends beyond the sterile compounding process. It involves ongoing consultations and discussions throughout the patient's treatment journey. The emphasis is on tailoring CSPs to meet the unique requirements of each patient. This patient-cantered approach ensures that medications are not only sterile and of high quality but also aligned with the individual patient's therapeutic needs (Jeyaraman *et al*., 2023).

3.5. Continuous professional development

The dynamic nature of pharmaceutical sciences requires pharmacists to engage in continuous professional development. Garcia and Patel (2019) emphasize the importance of ongoing education for pharmacists involved in sterile compounding. This involves staying abreast of new guidelines, technologies, and best practices, ensuring that pharmacists remain competent and capable of adapting to evolving standards.

Continuing education also plays a crucial role in addressing emerging challenges in sterile compounding. As highlighted by Garcia and Patel (2019), pharmacists who prioritize continuous learning are better equipped to navigate changes in regulations, incorporate new technologies, and implement best practices, ultimately contributing to the ongoing improvement of the sterile compounding process.

4. Conclusion

In conclusion, the pharmacist's role in sterile compounding is multifaceted and indispensable. Their commitment to regulatory compliance, patient safety, integration of technology, interdisciplinary collaboration, and continuous professional development collectively positions pharmacists as crucial contributors to the quality and safety of compounded sterile products. As the field continues to evolve, ongoing research, education, and collaboration are imperative to optimize the pharmacist's role in sterile compounding. The findings from various journals underscore the complexity of their responsibilities and emphasize the need for a holistic approach to ensure the integrity and safety of compounded sterile products in diverse healthcare settings.

References

Abdelaziz, H., Al Anany, R., Elmalik, A., Saad, M., Prabhu, K., Al-Tamimi, H., Salah, S. A., & Cameron, P. (2016). Impact of Clinical Pharmacy Services In A Short Stay Unit of A Hospital Emergency Department In Qatar. *International Journal of Clinical Pharmacy*, *38*(4), 776–779. https://doi.org/10.1007/s11096-016-0290-9

Abdulghani, K. H., Aseeri, M. A., Mahmoud, A., & Abulezz, R. (2017). The Impact of Pharmacist-Led Medication Reconciliation During Admission At Tertiary Care Hospital. *International Journal of Clinical Pharmacy*, *40*(1), 196–201. https://doi.org/10.1007/s11096-017-0568-6

Agomo, C. O., Ogunleye, J., & Portlock, J. (2016). Strategies Enhancing The Public Health Role of Community Pharmacists: A Qualitative Study. *Journal of Pharmaceutical Health Services Research*, *7*(2), 97–104. https://doi.org/10.1111/jphs.12131

Alhamoudi, A., & Alnattah, A. (2018). Pharmacy Education In Saudi Arabia: The Past, The Present, and The Future. *Currents in Pharmacy Teaching and Learning*, *10*(1), 54–60. https://doi.org/10.1016/j.cptl.2017.09.014

Aprilliano, B., Giuliano, C., Reed, J. B., & Kale-Pradhan, P. (2023). Evaluation of References Supporting Hospital Pharmacy Policies. *Medical Reference Services Quarterly*, *42*(1), 31–37. https://doi.org/https://doi.org/10.1080/02763869.2023.2160119

Ayalew, M. B., Solomon, L., & Abay, S. (2019). Pharmaceutical Care Practice in Community and Institutional Drug Retail Outlets of Gondar Town, North West Ethiopia. *Global Advances In Health and Medicine*, *8*, 1–9. https://doi.org/10.1177/2164956119868777

Brown, A., & White, B. (2021). Interdisciplinary Collaboration in Sterile Compounding: A Comprehensive Approach. *Journal of Pharmaceutical Practice, 29*(4), 123-140.

Croft, H., Gilligan, C., Rasiah, R., Levett-Jones, T., & Schneider, J. (2018). Thinking in Pharmacy Practice: A Study of Community Pharmacists' Clinical Reasoning in Medication Supply Using the Think-Aloud Method. *Pharmacy*, *6*(1), 1–14. https://doi.org/10.3390/pharmacy6010001

Dalton, K., & Byrne, S. (2017). Role of The Pharmacist In Reducing Healthcare Costs: Current Insights. *Integrated Pharmacy Research and Practice*, *6*, 37–46. https://doi.org/10.2147/iprp.s108047

EAHP. (2023). *LinkedIn Profile*. European Association Hospital Pharmacy.

https://be.linkedin.com/company/the-european-association-of-hospital-pharmacists

Fan, M., Yang, D., Ng, B., Jackson, J., Bouris, K., Eng, S., Rolko, E., & Trbovich, P. (2022). Impact of Technology-Assisted Versus Manual Sterile Compounding on Safety and Efficiency In A Canadian Community Hospital. *American Journal of Health-System Pharmacy*, *79*(19), 1685–1696. https://doi.org/10.1093/ajhp/zxac167

Hanifah, S., Ball, P. A., & Kennedy, R. A. (2022). Management of Y-Site Incompatibility of Intravenous Medication: A Scoping Review. *Indonesian Journal of Pharmacy*, *33*(3), 353–365. https://doi.org/10.22146/ijp.2262

Hanifah, S., Maulidani, Y., Nugroho, B. H., & Sari, C. P. (2021). Provision of Standardized All-in-One Parenteral Nutrition (AIO-PN) for Very Preterm Neonates: Evaluation at Room and Cold Temperatures. *Journal of Pharmacy and Bioallied Sciences*, *13*(1), 26–30. https://doi.org/10.4103/jpbs.JPBS

Helgesen, A. K., Nome, C. M., Stenbjerg, J. K., Arnesen, M. H., Aardalen, T. R., Dilles, T., & Grøndahl, V. A. (2024). Pharmaceutical Care in Primary Healthcare—A Study of Nurses', Pharmacists', and Physicians' Experiences of Interprofessional Collaboration. *Healthcare*, *12*(1100), 1–12. https://doi.org/10.3390/healthcare12111100

Islam, M. A., Khan, S. A., Gunaseelan, S., & Talukder, R. (2015). Specialty Education For Student Pharmacists and PharmD Graduates In U.S. Colleges and Schools of Pharmacy. *Currents in Pharmacy Teaching and Learning*, *8*(2), 184–190. https://doi.org/10.1016/j.cptl.2015.12.001

Jean, S. J., Francart, S. J., Eckel, S. F., Schenkat, D., Eberwein, S., Lamm, M., Barnes, N., Valgus, J. M., & Amerine, L. B. (2020). Evaluation of Telepharmacy and The Use of A Gravimetric Technology–Assisted Workflow System For Remote Sterile Product Pharmacist Checks. *American Journal of Health-System Pharmacy*, *77*(7), 560–567. https://doi.org/10.1093/ajhp/zxaa015

Jeyaraman, M., Ramasubramanian, S., Kumar, S., Jeyaraman, N., Selvaraj, P., Nallakumarasamy, A., Bondili, S. K., & Yadav, S. (2023). Multifaceted Role of Social Media In Healthcare: Opportunities, Challenges, and The Need For Quality Control. *Cureus*, *15*(5), e39111. https://doi.org/https://doi.org/10.7759/cureus.39111

Johnson, R., & Davis, P. (2020). Ensuring Patient Safety in Sterile Compounding: The Pharmacist's Imperative. *Pharmacy Today, 26*(5), 45-52.

Kemenkes RI. (2016). *Peraturan Menteri Kesehatan Republik Indonesia No. 72 Tahun 2016 tentang Pekerjaan Kefarmasian di Rumah Sakit*. Jakarta: *K*ementrian Kesehatan Republik Indonesia.

Kim, E., Worley, M. M., & Law, A. V. (2023). Pharmacist Roles In The Medication Use Process: Perceptions of Patients, Physicians, and Pharmacists. *Journal of the American Pharmacists Association*, *63*(4), 1120–1130.

Garcia, M., & Patel, S. (2019). Continuous Professional Development for Pharmacists in Sterile Compounding: Navigating Evolving Standards. American Journal of Health-System Pharmacy, 76(17), 1325-1334. https://doi.org/10.1016/j.japh.2023.05.013

Lam, S. W., & Sokn, E. (2019). Effect of Pharmacy-Driven Bedside Discharge Medication Delivery Program on Day 30 Hospital Readmission. *Journal of Pharmacy Practice*, *33*(5), 628–632. https://doi.org/10.1177/0897190019825961

Lee, K., O'Donnell, L. K., Cross, A. J., Hawthorne, D., & Page, A. T. (2023). Clinical Pharmacists' Reported Approaches and Processes For Undertaking Home Medicines Review Services: A National Survey. *Archives of Gerontology and Geriatrics*, *109*, 104965. https://doi.org/https://doi.org/10.1016/j.archger.2023.104965

Lee, C., & Chen, L. (2018). Integrating Automation in Sterile Compounding: A Review of Technological Advancements. *Journal of Pharmacy Technology, 34*(2), 67-75.

Mill, D., Johnson, J. L., Lee, K., Salter, S. M., D'Lima, D., Seubert, L., Clifford, R., & Page, A. T. (2021). Use of Professional Practice Guidance Resources In Pharmacy: A Cross-Sectional Nationwide Survey of Pharmacists, Intern Pharmacists, and Pharmacy Students. *Journal of Pharmaceutical Policy and Practice*, *14*(114), 1–19. https://doi.org/10.1186/s40545-021-00395-8

Minor, S., Huffman, M., Lewis, P., Kost, A., & Prunuske, J. (2019). Community Preceptor Perspectives on Recruitment and Retention: The CoPPRR Study. *Family Medicine*, *51*(5), 389–398. https://doi.org/10.22454/FamMed.2019.937544

Mustikawati, Hendrawati, Rahayu, W., & Karim, A. (2023). Effective Communication on Adolescent Delinquency at SMK Jaya Buana. *Journal of Community Engagement in Health*, *6*(1), 73–78. https://doi.org/10.30994/jceh.v6i1.371

Myers, C. E. (2013). History of Sterile Compounding In U.S. Hospitals: Learning From The Tragic Lessons of The Past. *American Journal of Health-System Pharmacy*, *70*(16), 1414–1427. https://doi.org/10.2146/ajhp130112

Qadus, S., Naser, A. Y., Al-Rousan, R., & Daghash, A. (2022). Utilization of Drug Information Resources Among Community Pharmacists In Jordan: A Cross-Sectional Study. *Saudi Pharmaceutical Journal*, *30*(1), 1–7. https://doi.org/10.1016/j.jsps.2021.12.001

Saseen, J. J., Ripley, T. L., Bondi, D., Burke, J. M., Cohen, L. J., McBane, S., McConnell, K. J., Sackey, B., Sanoski, C., Simonyan, A., Taylor, J., & Griend, J. P. Vande. (2017). ACCP Clinical Pharmacist Competencies. *Pharmacotherapy*, *37*(5), 630–636. https://doi.org/10.1002/phar.1923

Smith, J., et al. (2019). Regulatory Compliance in Sterile Compounding: Navigating USP and FDA Guidelines. *International Journal of Pharmaceutical Compounding, 23*(1), 34-45.

Tran, T., Khattar, S., Vu, T. T., Potter, M., Hodding, J., Kuo, G. M., & Le, J. (2017). Impact of Pharmacist Discharge Counseling On Hospital Readmission and Emergency Department Visit. *Journal of Hospital Administration*, *6*(2), 68–73. https://doi.org/10.5430/jha.v6n2p68

Truong, H., & Nguyen, T. (2020). An Assessment of Pharmacist's Sterile Compounding Competencies. Journal of Pharmacy Practice, 0897190020969144

United States Pharmacopeia (USP). (2023). Standards Build Trust in Medicines and a Stronger Supply Chain. Available from https://www.usp.org/

Whyte, W. (2004). Assessing Microbial Risk To Patients From Aseptically Manufactured Pharmaceuticals. *European Journal of Parenteral and Pharmaceutical Sciences*, *9*(3), 71–77.

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α-amylase inhibition test of ethanol extract colues leaves (*Solenostemon scutellarioides* (L.) Codd) *in vitro*

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Abstract

Background: Hyperglycemia occurs due to an increase in blood glucose levels, making them higher than the normal range, which is a sign of diabetes mellitus. One therapeutic approach for DM is inhibiting enzymes that convert carbohydrates into glucose. Coleus (*S. scutellarioides* (L.) Codd), an ornamental plant, is believed to have antihyperglycemic effects due to its phenolic phytochemicals.

Objective: To determine the phytochemical content and antihyperglycemic activity of 70% ethanol extract of coleus leaves through α-amylase enzyme inhibition.

Method: The leaves were characterized and tested qualitatively for phytochemical content using the test tube method. The extract was prepared using maceration with 70% ethanol, followed by thin-layer chromatography (TLC). The 200-ppm extract was tested for α-amylase enzyme inhibition, with acarbose as a positive control. Statistical differences were analyzed using the t-test method.

Results: The herbarium specimen of coleus leaf was shown to contain flavonoids, tannins, saponins, and steroids. The 200-ppm extract reduced the activity of the α-amylase enzyme by 61.03%, while the positive control (acarbose) reduced it by 92.16%.

Conclusion: The coleus leaf extract contains active phytochemicals that may help lower blood glucose levels. At 200 ppm, the extract demonstrates potential as an antihyperglycemic agent through α-amylase inhibition.

Keywords: coleus leaves, antihyperglycemic, α-amylase enzymes inhibition

1. Introduction

Diabetes mellitus (DM), a serious metabolic disease is characterized by hyperglycemia, a state where blood glucose level is higher than the normal concentrations. It is a metabolic disease initiated by a defect in insulin production or action, where insulin is the hormone that plays a role in regulating blood glucose levels (Kemenkes RI, 2020). Common strategies for managing DM include increasing the sensitivity of insulin receptors, administering insulin therapy, and inhibiting glucose absorption through the digestive system. One therapeutic approach which has been studied extensively concerning the management of DM is the inhibition of digestive enzymes such as α-amylase, which is responsible for the hydrolysis of complex carbohydrates into glucose. By inhibiting this enzyme, intake of glucose in the body post meals can be reduced which may alleviate hyperglycemic conditions on patients with DM (Kemenkes RI, 2020).

The use of natural products as alternative therapies, particularly in the management of chronic diseases such as DM, has received widespread attention. This is because many natural substances contain bioactive compounds like flavonoids, alkaloids, tannins, and saponins that have pharmacological properties, including antihyperglycemic effects (Grover *et al*., 2002). Several studies have reported that these phytochemicals are capable of inhibiting certain digestive enzymes, such as α-amylase, thereby contributing to the reduction of blood glucose levels (Tundis *et al*., 2010). One of

the plants with potential antihyperglycemic effects that is commonly cultivated among the Indonesian communities is the coleus (*S. scutellarioides* (L.) Codd).

Despite being frequently grown as decorative plants, coleus leaves may also have the potential to lower blood sugar levels, which lowers the risk of developing DM (Lestari, 2022). Numerous chemical substances, such as flavonoids, saponins, tannins, and steroids, have been discovered to be present in the herbarium specimen (dried plant material) of coleus leaves (Natasya *et al*., 2023). However, verification of the phytochemical content of plants is trill required, because the presence and concentration of bioactive compounds can be affected by plants parts, growing conditions, and harvest time (Azwanida, 2015; Verpoorte *et al*., 2000). Since there is limited research on the potential of coleus leaves, *in vitro* approaches are also required to evaluate the antihyperglycemic activity of these compounds. Thus, the purpose of this study was to determine which secondary metabolites were found in the herbarium specimen and extracts, as well as to examine the 70% ethanol extract of coleus leaves' ability to inhibit α-amylase enzyme.

2. Method

2.1. Type and design of study

This was an experimental study using a 70% ethanol extract of coleus leaves at a concentration of 200 ppm, with acarbose as the positive control at a concentration of 100 ppm.

2.2. Population and sample

The coleus leaves used in this study were collected from Sukorejo, Gunungpati Regency, Semarang City. The inclusion criteria for coleus leaves were: green and purplish-red coloration, the third to the eighth leaves from the tip, harvested in the morning, fresh, intact without holes or mold, and uniform in size.

2.3. Tools and materials

Tools used in this research included a blender (Philips), glassware (Pyrex), 40-mesh sieve (CBN), test tubes (Iwaki), micropipettes (Socorex), analytical balance (Mettler Toledo, USA), magnetic stirrer (Benchmark), incubator (Fisher), UV-VIS spectrophotometer (Shimadzu), microscope (Olympus, China), microscope slides (Sail Brand), water bath, furnace, rotary vacuum evaporator, sieve, dehydrator, vortex (Boeco V-1 plus), cuvettes, stationery, scissors, gloves, silica gel GF254 plates (Merck), and chromatography chamber.

Materials used included dried coleus leaves from Sukorejo, Gunungpati Regency, Semarang City; distilled water; 70% ethanol (p.a Merck); acarbose (p.a Merck); methanol (p.a Smartlab); chloroform (p.a Smartlab); concentrated HCl (p.a Smartlab); ethyl acetate (p.a Smartlab); acetic anhydride (p.a Smartlab); Mayer's reagent; Liebermann-Burchard reagent; Dragendorff's reagent (p.a Merck); α-amylase enzyme (fermentation product from B. licheniformis); amylase activity assay kit (Sigma-Aldrich); magnesium powder (p.a Merck); sulfuric acid (p.a Tian Jin Shin Damao); ferric chloride (p.a Smartlab); aluminum chloride (p.a Smartlab); piperine; gallic acid; cholesterol; quercetin (p.a Smartlab); water for injection (p.a Smartlab); and aluminum foil (local).

2.4. Plant identification

Coleus leaves were identified by comparing the plant parts to the morphological characteristics of *S. scutellarioides* (L.) Codd. The identification process was conducted at the Laboratory of Ecology and Biosystematics, Faculty of Science and Mathematics, Diponegoro University, Semarang, confirming that the sample was classified as the order Lamiales and the species *Solenostemon scutellarioides* (L.) Codd.

2.5. Preparation of herbarium specimen

A total of 1 kg of fresh coleus leaves was collected, washed thoroughly, and drained. The leaves were then sorted, sliced, and dried using a dehydrator at 40–45°C for 2–3 days until fully dry (crisp-dry). The dried leaves were ground into powder using a grinder and standardized using a 40-mesh sieve, yielding 100 grams of dried powder.

2.6. Characterization of herbarium specimen

2.6.1. Macroscopic examination of fresh coleus leaves

This test aimed to identify the distinctive features of the coleus leaves through direct observation of its characteristics, referring to the morphology of coleus leaves, including shape, odor, and color (Kemenkes RI, 2017).

2.6.2. Microscopic examination of herbarium specimen

A thin transverse section of the coleus leaf was prepared and observed under a microscope by placing the section on a slide with a drop of water, covered with a coverslip. The same method was applied to the coleus leaves powder. The specimens were observed under 40× magnification (Kemenkes RI, 2017).

2.6.3. Ethanol-soluble extract content

Five grams of the coleus leaves powder were macerated for 24 hours in 100 mL of 70% ethanol using a stoppered flask, shaken frequently during the first 6 hours, and then left to stand for 18 hours before filtration. Twenty milliliters of the filtrate were evaporated using a water bath in a pre-heated porcelain dish (105°C) and weighed until a constant weight was achieved (Kemenkes RI, 2017).

2.6.4. Water-soluble extract content

Five grams of coleus leaves powder were macerated in 100 mL of water containing chloroform (2.5 mL of chloroform in 1 L of distilled water) using a stoppered flask, shaken for the first 6 hours, and then allowed to stand for 18 hours before filtration. Twenty milliliters of the filtrate were evaporated using a water bath in a pre-heated porcelain dish (105°C) and weighed until a constant weight was obtained (Kemenkes RI, 2017).

2.6.6. Loss on drying

Two grams of coleus leaves powder were accurately weighed and placed in a porcelain crucible pre-heated at 105°C for 30 minutes and pre-weighed. The crucible was placed in an oven with the lid open and dried at 105°C until constant weight, then cooled in a desiccator and weighed again (Kemenkes RI, 2017).

2.6.6. Moisture content

Ten grams of coleus leaves powder were weighed and placed in a tared container, then 200 mL of saturated toluene was added to the flask containing the test sample and heated. Distillation was carried out at a rate of approximately two drops per second until the water was completely distilled. The volume of distilled water was read using the scale on the distillation apparatus (Kemenkes RI, 2017)

2.6.7. Total ash content

Two grams of coleus leaves powder were weighed and placed in a tared, pre-ignited porcelain crucible. The crucible containing the sample was gradually ignited until the carbon was completely burned off. The ignition was performed at 600°C for 3 hours, then cooled and weighed until a constant weight was obtained (Kemenkes RI, 2017).

2.6.8. Acid-insoluble ash content

The ash obtained from the total ash determination was treated with 25 mL of dilute hydrochloric acid for 5 minutes. The acid-insoluble residue was filtered through ash-free filter paper, washed with hot water, ignited until a constant weight was achieved, and then weighed (Kemenkes RI, 2017).

2.7. Sample extraction

A total of 100 g of dried coleus powder was weighed and macerated in a dark-colored glass container with 70% ethanol as the solvent, using a coleus powder-to-solvent ratio of 1:10. The container was sealed and stored at room temperature, protected from light for 3 × 24 hours, while being stirred frequently during the first 6 hours. Filtration was performed every 24 hours, followed by re-maceration using fresh solvent. The resulting filtrate was evaporated using a rotary evaporator at 40°C until a thick extract was obtained.

2.8. Phytochemical screening of herbarium specimen

2.8.1. Flavonoids

One gram of coleus leaves powder was added to 10 mL of distilled water, boiled for 5 minutes, and then filtered while hot. Five mL of the filtrate was added with magnesium powder and 1 mL of concentrated hydrochloric acid. The presence of flavonoids is indicated by the formation of an orange, red, or yellow color (Farnsworth, 1966).

2.8.2. Terpenoids and steroids

A total of 0.5 grams of coleus leaves powder was macerated with 10 mL of n-hexane for 1 hour, then filtered. Two mL of the filtrate was added with 1 mL of acetic anhydride. The mixture was then carefully layered with 2 mL of concentrated sulfuric acid along the wall of the test tube. A blue, purple, or green color indicates the presence of steroids, while a red or brown color indicates the presence of triterpenoids (Farnsworth, 1966).

2.8.3. Tannins

One gram of coleus leaves powder was added to 10 mL of water, boiled, and filtered. A few drops (1–2) of ferric chloride reagent were added to the filtrate. A blue-black or greenish-black coloration indicates the presence of tannins (Farnsworth, 1966).

2.8.4. Saponins

A total of 0.5 grams of coleus leaves powder was placed into a test tube and added with 10 mL of hot water, then allowed to cool and shaken for 10 seconds. The formation of persistent foam for no less than 10 minutes at a height of 1–10 cm that does not disappear upon the addition of one drop of 2 N hydrochloric acid indicates the presence of saponins (Farnsworth, 1966).

2.8.5. Alkaloids

A total of 0.5 grams of coleus leaves powder was added with 1 mL of hydrochloric acid and 9 mL of distilled water, then heated for 2 minutes, cooled, and filtered. The filtrate was used for alkaloid testing. 0.5 mL of the filtrate was distributed into three separate test tubes. Test tube 1 was added

with Mayer's reagent, test tube 2 was added with Dragendorff's reagent, and test tube 3 was added with Wagner's reagent. The presence of alkaloids is indicated by a white precipitate in test tube 1, a brown precipitate in test tube 2, and a brick-red precipitate in test tube 3 (Farnsworth, 1966).

2.9. Phytochemical screening of the extract

Phytochemical tests using Thin Layer Chromatography (TLC) were carried out on compound groups that showed positive results in the phytochemical screening using reagent-based methods. The stationary phase used was silica gel G60F254/TLC plates.

2.9.1. Identification of flavonoid compounds

The 70% ethanol extracts of the coleus leaves, and a reference standard (quercetin) were spotted using a capillary tube at a distance of 1 cm from the bottom edge of the silica gel GF254 TLC plate, which had been previously heated in an oven at 110°C for approximately 30 minutes. After the spots dried, the TLC plate was placed in a chromatography chamber containing a pre-saturated mobile phase of chloroform:ethyl acetate (8.5:1.5). Elution was carried out until it reached the upper limit mark. The eluted TLC plate was then observed under UV light at λ254 and λ366, both before and after being sprayed with 10% $AlCl_3$. Detected spots were analyzed for their Rf values and observed for their color appearances (Farnsworth, 1966).

2.9.2. Identification of steroid compounds

The 70% ethanol extracts of the coleus leaves, and a reference standard (cholesterol) were spotted using a capillary tube at a distance of 1 cm from the bottom edge of the silica gel GF254 TLC plate, which had been previously heated in an oven at 110°C for approximately 30 minutes. After the spots dried, the TLC plate was placed in a chromatography chamber containing a pre-saturated mobile phase of chloroform:ethyl acetate (8.5:1.5). Elution was carried out to the upper limit mark. The eluted TLC plate was then observed under UV light at λ254 and λ366, both before and after being sprayed with Liebermann-Burchard reagent. Detected spots were analyzed for their Rf values and observed for their color appearances (Farnsworth, 1966).

2.9.3 Identification of Tannin Compounds

The 70% ethanol extracts of the coleus leaves, and a reference standard (gallic acid) were spotted using a capillary tube at a distance of 1 cm from the bottom edge of the silica gel GF254 TLC plate, which had been previously heated in an oven at 110°C for approximately 30 minutes. After the spots dried, the TLC plate was placed in a chromatography chamber containing a pre-saturated mobile phase of chloroform:ethyl acetate (8.5:1.5). Elution was carried out to the upper limit mark. The eluted TLC plate was then observed under UV light at λ254 and λ366, both before and after being

sprayed with $FeCl_3$. Detected spots were analyzed for their Rf values and observed for their color appearances (Farnsworth, 1966)

2.9.4. Identification of alkaloid compounds

The 70% ethanol extract of the coleus leaves, and a reference standard (piperine) were spotted using a capillary tube at a distance of 1 cm from the bottom edge of the silica gel GF254 TLC plate, which had been previously heated in an oven at 110°C for approximately 30 minutes. After the spots dried, the TLC plate was placed in a chromatography chamber containing a pre-saturated mobile phase of chloroform:ethyl acetate (8.5:1.5). Elution was carried out to the upper limit mark. The eluted TLC plate was then observed under UV light at λ254 and λ366, both before and after being sprayed with Dragendorff reagent. Detected spots were analyzed for their Rf values and observed for their color appearances (Farnsworth, 1966).

2.10. Preparation of test solutions

2.10.1. Enzyme stock solution

The enzyme stock solution was prepared at a concentration of 10,000 ppm. α-Amylase enzyme (50 mg) was weighed and dissolved in assay buffer solution up to a volume of 5 mL, then vortexed for 30 seconds until homogeneous. The α-amylase enzyme stock solution at 10,000 ppm is equivalent to 204.20 U/mL of solution.

2.10.2. Acarbose stock solution

Acarbose solution was prepared at a concentration of 1000 ppm. Acarbose powder (50 mg) was weighed, dissolved in 50 mL of water for injection, and vortexed for 30 seconds until homogeneous.

2.10.3. Master mix solution

The master reaction mix was prepared by mixing 25 µL of buffer and 25 µL of substrate, then homogenized using pipetting technique.

2.10.4. Sample stock solution

The extract stock solution was prepared at a concentration of 1000 ppm. The extract (50 mg) was weighed, dissolved in 50 mL of water for injection, and vortexed for 30 seconds until homogeneous.

2.11. Enzyme optimization and determination of operating time

Enzyme optimization was performed by reacting several variations of α-amylase enzyme units with 25 µL of substrate. The optimal enzyme unit was determined based on the amount of

enzyme that could hydrolyze 25 µL of substrate within a specific period. The time required to fully hydrolyze the substrate was used as the operating time for the assay. The optimization test was conducted by reacting the enzyme solution with 50 µL of the master mix (substrate + buffer). The mixture was incubated for 2 minutes, and absorbance was measured at λ 405. The mixture was then incubated at 25°C, and absorbance was measured every 5 minutes using a UV-Vis spectrophotometer at λ 405 until a constant absorbance was reached (Hidayah *et al*., 2023).

2.12. α-Amylase activity assay

2.12.1. Positive control

The positive control test was conducted by reacting 125 µL of α-amylase enzyme solution (25 U/mL) with 75 µL of acarbose solution at a concentration of 100 ppm, followed by the addition of 50 µL of the master mix. The solution mixture was homogenized vertically using pipetting technique. The mixture was incubated for 2 minutes, and absorbance was measured at λ 405. It was then incubated at 25°C and measured again using a UV-Vis spectrophotometer at λ 405. The test was performed in triplicate (Fathiyah, 2023).

2.12.2. Sample

The sample test was conducted by reacting 125 µL of α-amylase enzyme solution (25 U/mL) with 75 µL of the coleus leaves extract sample solution at a concentration of 200 ppm, followed by the addition of 50 µL of the master mix. The solution mixture was homogenized vertically using pipetting technique. The mixture was incubated for 2 minutes, and absorbance was measured at λ 405. It was then incubated at 25°C and measured again using a UV-Vis spectrophotometer at λ 405. The test was performed in duplicate (Fathiyah, 2023).

3. Result and discussion

3.1. Characterization of coleus (S. scutellarioides (L.) Codd) leaves

Macroscopic analysis of the powdered dried coleus leaf reveled that the leaf, which was 9 cm long, was reddish-purple in the middle and green around the edges. The leaf blade had pinnate venation, serrated edges, and a pointed tip. It was ovate in shape. Microscopic examination of the transverse section of the coleus leaf showed the presence of covering trichomes, cuticle, palisade cells, epidermis, glandular trichomes, vascular bundles, and collenchyma. The leaves powder's microscopic analysis indicated the presence of epidermal cells, glandular trichomes, and covering trichomes. **Table 1** displays the findings for the dried coleus leaf powder's moisture content, water-

soluble extractive value, ethanol-soluble extractive value, total ash content, and acid-insoluble ash content.

Table 1. Characterization of dried coleus (*S. scutellarioides* (L.) codd) leaves powder

Parameter	Result (% ± SD)	FHI literature standard (%)
Drying loss	7.53±0.31	≤ 10
Ethanol-soluble extract content	14.83±0.73	≥ 5
Water-soluble extract content	22.91±0.72	≥ 22
Moisture content	5.67±0.58	≤ 10
Total ash content	5.46±0.17	≤ 8
Acid-insoluble ash content	0.61±0.09	≤ 2

According to **Table 1**, the coleus leaves powder's characterization complied with the Indonesian Herbal Pharmacopeia's (FHI) and *Materia Medika Indonesia's* (FHI) standards. The amounts of compounds in the crude drug that can dissolve in ethanol and water were ascertained by testing the coleus leaves powder's contents that were soluble in ethanol and water, respectively. The water-soluble extract content was higher than the ethanol-soluble extract content, indicating that the active compounds in the coleus leaves are better extracted in water than in ethanol (Depkes, 2000).

Moisture content is a necessary parameter to test, as it is related to the quality of the crude drug during storage. Over time, a high moisture content can inhibit the biological activity of the crude drug and encourage the growth of mold (Depkes, 2000). The purpose of total ash content test was to determine the amount of internal and external mineral matter was produce during the processing of crude drugs. Ash can originate from outside pollutants or the plant itself. Finding out how much ash comes from outside source like soil and sand is the goal of the acid-insoluble ash test (Depkes, 2000).

3.2. Extraction of coleus (S. scutellarioides (L.) Codd) leaves

The extraction of the coleus leaves was carried out using 70% ethanol as the solvent. The 70% ethanol solvent aimed to extract both polar and non-polar compounds (Riwanti *et al.*, 2020). The coleus leaves extracted using the 70% ethanol obtained was dark brown in color, with a yield of 13.67 g from 100 g of the sample, resulting in an extraction yield of 13.67%. This yield was higher than that reported in a previous study, which used the same solvent, i.e., 12.97% (Susilawati *et al*., 2016). The difference in yield may be due to differences in the extraction methods. The previous study did not replace the solvent during the maceration process, which led to fewer active compounds being extracted, as the solvent had become saturated.

3.3. Phytochemical content of herbarium specimen of coleus (S. scutellarioides (L.) Codd) leaves

Phytochemical screening aims to identify the content of secondary metabolite compounds in the herbarium specimen. The results of the phytochemical screening can be seen in **Table 2**.

Table 2. Phytochemical content of herbarium specimen of coleus *(S. scutellarioides (L.) codd)* leaves

Compoud	Control	Reagent	Literature	Result	Description
Alkaloids	Piperine	Mayer	White precipitate	Yellow	-
		Dragendorff	Brown precipitate	Black	-
		Wagner	Brick-red precipitate	Black	-
Flavonoids	Quercetin	HCl + Mg	Red, yellow, or orange	Yellow	+
Tannins	Gallic Acid	Water + $FeCl_3$	Blue or dark green	Dark green	+
Saponins	Sapogenin	Air panas	Foam formed	Foam formed	+
Steroids	Cholesterol	Lieberman Burchard	Blue, purple, or green	Green	+

Description:
(+): Contains compound mentioned
(-): Does not contain compound mentioned

The finding of this study's phytochemical content were in line with earlier investigations that discovered secondary metabolites like flavonoids, tannins, steroids, and saponins in the coleus leaves herbarium specimen (Susilawati *et al*., 2016). The natural material's chemical content should be re-examined in light of variations in sample collection locations, harvest times, and plant parts examined, all of which may have an impact on the presence and concentration of the material's bioactive compounds (Azwanida, 2015; Verpoorte *et al*., 2000). To confirm that alkaloid compounds were present in the coleus leaves herbarium specimen, the alkaloid test was carried out three times. Since all three reagents produced negative results, it is likely that coleus leaves's low alkaloid content prevented it from being picked up during the phytochemical screening. The existence of these secondary metabolites raises the possibility that coleus leaves could have pharmacological effects and be used as a medicinal ingredient, especially for antidiabetic purposes. The amylase enzyme is known to be inhibited by flavonoids, tannins, saponins, and steroids (Wahyuntari, 2011; Wakhidah & Silalahi, 2018).

3.4. Identification of secondary metabolite compounds by thin layer chromatography

The identification of secondary metabolite compounds in the 70% ethanol extract of coleus leaves was performed using TLC with a stationary phase of silica gel GF254 and a mobile phase of chloroform:ethyl acetate (8.5:1.5). Based on the eluents, a TLC profile was created using specific spot indicators, namely $AlCl_3$ as a specific spot indicator for flavonoids, $FeCl_3$ for tannins, Lieberman Burchard for steroids, and Dragendorff for alkaloids.

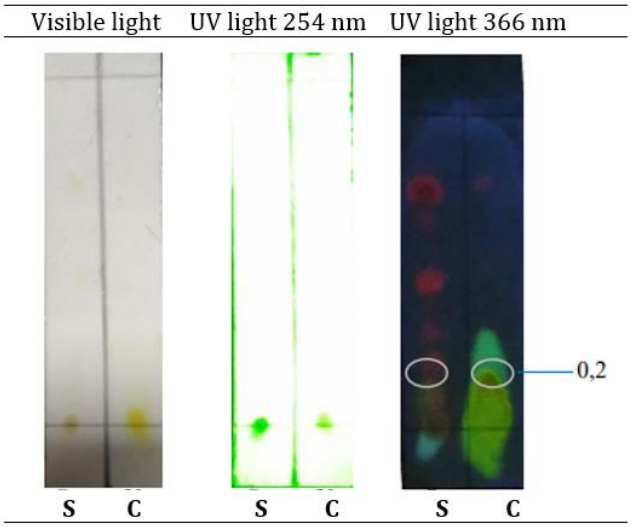

Figure 1. TLC profile of flavonoid compounds in 70% ethanol extract of coleus leaves (*S. scutellarioides* (L.) Codd) after spraying with $AlCl_3$ spot reagent; (S) sample (C) quercetin as a control

Figure 1 shows a yellow-green spot on the sample, indicating the presence of flavonoid compounds in the 70% ethanol extract of coleus leaves with an Rf value of 0.2. This is consistent with a study by (Lisdawati *et al.*, 2012), which also showed the presence of flavonoid compounds in the coleus leaves extract, even though the sample was collected from a different growing location. The use of the specific spot reagent $AlCl_3$ aimed to react with the spot by spraying it onto the TLC plate, making the spot visible under UV light at λ 366. The identification of flavonoid compounds by TLC reinforced the results of the phytochemical screening of herbarium specimen of the coleus leaves.

The presence of tannin compounds in the 70% ethanol extract of coleus leaves was indicated by a yellow-green spot on the sample in **Figure 2**. This finding in line with the previous study, which also showed the presence of tannin compounds in the coleus leaves extract (Lisdawati *et al*., 2012). By spraying the spot onto the TLC plate, the specific spot reagent $FeCl_3$ was used to react with the spot and make it visible under UV light at λ 366. The findings of the phytochemical screening of the coleus leaves herbarium specimen were supported by the TLC identification of tannin compounds.

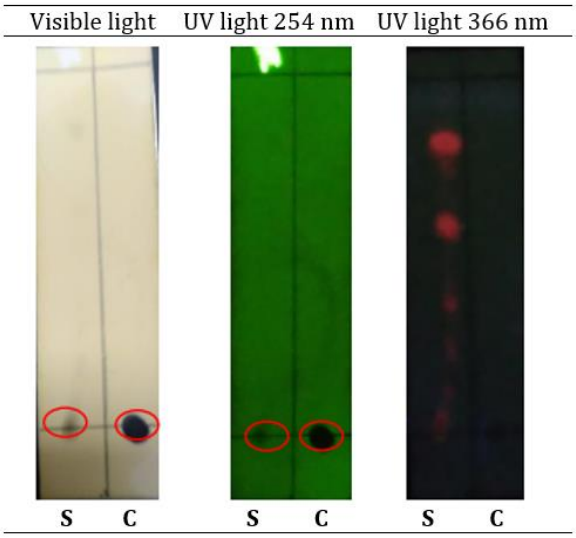

Figure 2. TLC profile of flavonoid compounds in 70% ethanol extract of coleus leaves (*S. scutellarioides* (L.) Codd) after spraying with $FeCl_3$ spot reagent; (S) sample (C) gallic acid as a control

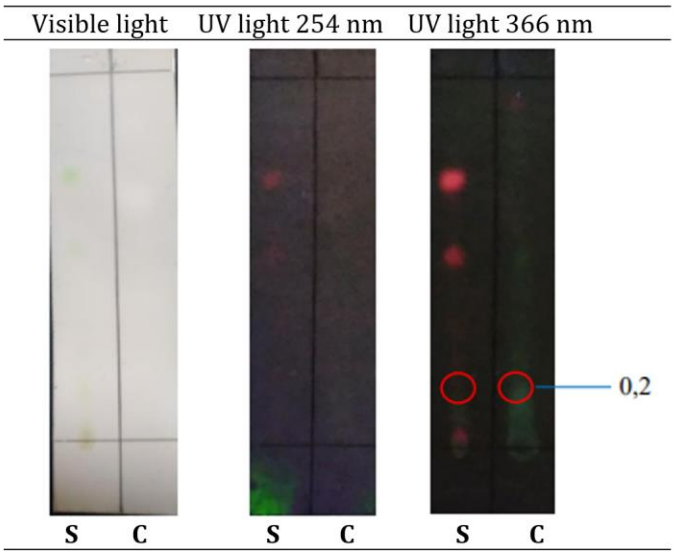

Figure 3. TLC profile of flavonoid compounds in 70% ethanol extract of coleus leaves (*S. scutellarioides* (L.) Codd) after spraying with Lieberman Burchard spot reagent; (S) sample (C) cholesterol as a control

Figure 3 shows a bluish-green spot on the sample, indicating the presence of steroid compounds in the 70% ethanol of coleus leaves with an Rf of 0.2. This is consistent with a study by (Lisdawati *et al*., 2012), which also showed the presence of steroid compounds in the extract of miana leaves. The use of specific spot reagent Lieberman Burchard aimed to react with the spot by spraying it onto the TLC plate, making the spot visible under UV light at λ366. The identification of steroid compounds by TLC strengthened the results of the phytochemical screening of the herbarium specimen of the coleus leaves.

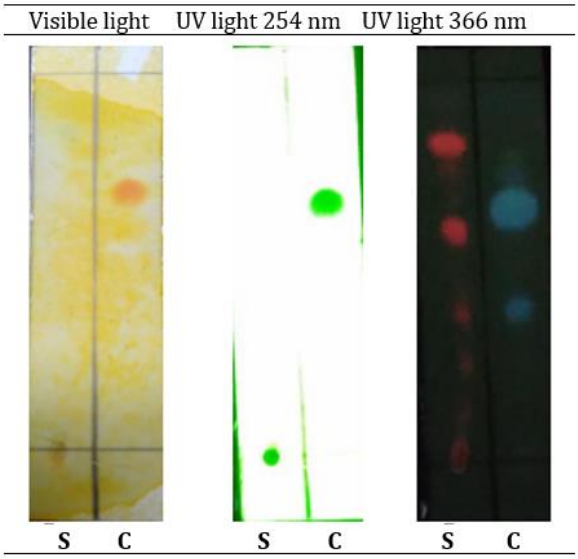

Figure 4. TLC profile of flavonoid compounds in 70% ethanol extract of coleus leaves (*S. scutellarioides* (L.) Codd) after spraying with Dragendorff spot reagent; (S) sample (C) piperine as a control

The lack of yellow spot on the sample in the **Figure 4** suggests that the 79% ethanol of coleus leaves does not certain any alkaloid compounds. This is consistent with previous study, which also demonstrated the absence of alkaloid compounds in the coleus leaves extract (Lisdawati *et al*., 2012). The use of specific spot reagent Dragendorff aimed to react with the spot by spraying it onto the TLC plate, making the spot visible under UV light at λ 366. The alkaloid test by TLC gave a negative result, because the 70% ethanol of coleus leaves contains a very little quantity of alkaloid, making it undetectable. The identification of alkaloid compounds by TLC supported the results of the herbarium specimen of the coleus leaves.

3.5. α-Amylase enzyme inhibition test

The average % inhibition of the α-amylase enzyme by 100 ppm acarbose and 200 ppm of the 70% ethanol of coleus leaves can be seen in **Table 3**.

Table 3. Percentage of α-amylase enzyme inhibition by 70% ethanol extract coleus leaves (*S. scutellarioides* (L.) Codd)

Sample	Average inhibition (%± SD)	Sig. (2-tailed)
70% ethanol extract of coleus leaves at 200 ppm	61.03±1.04	0.043
Positive control (acarbose) at 100 ppm	92.16±0.85	

The 70% ethanol extract of coleus leaves was able to inhibit the α-amylase enzyme with an inhibition percentage of 61.03%. This indicates that coleus leaves extract has potential as an anti-hyperglycemic agent because it can inhibit enzymes that metabolize carbohydrates, with an α-amylase enzyme inhibition percentage exceeding 50%. In the meantime, 92.16% of the α-amylase enzyme could be inhibited by acarbose as the positive control. The t-test method of statistical analysis

revealed that the percentage of enzyme inhibition by the positive control, acarbose, was higher than that of the coleus leaves extract, and that the difference between the two was statistically significant (p<0.05). This implies that in order to attain more ideal enzyme inhibition, a higher concentration of coleus leaves extract might still be required.

In a different *in vivo* study, mice given alloxan showed the greatest decrease in blood glucose percentage when given coleus leaves at a dose of 200 mg/kg BW. Due to the paucity of research on the *in vitro* antihyperglycemic activity of coleus leaves extract, the use of 200 ppm concentration of coleus extract leaves in this study was based on this *in vivo* previous research, which used 200 mg/kg BW *in vivo* (Lestari, 2022). The results of this study indicate that the 200-ppm concentration of coleus leaves extract, which inhibit 61.03% of α-amylase, can still be increased to achieve the optimal inhibition enzyme responsible for carbohydrate lysis.

The 70% ethanol extract of coleus leaves contained flavonoids, tannins, saponins, and steroids, according to the phytochemical screening and TLC profile analysis. These bioactive compounds may be the reason for the inhibition mechanism of coleus leaves on α-amylase because they bind to the enzyme's active site and prevent it from interacting with its substrate, starch, which inhibits the breakdown of starch into glucose and lowers blood sugar levels. These bioactive compound act as competitive inhibitors of the enzyme because they have similar structures to substrate and compete to occupy the enzyme's active site, thereby inhibiting α-amylase (Nafiu & Tom Ashafa, 2017; P *et al*., 2011; Proença *et al*., 2019).

The α-amylase enzyme plays a key role in the early stages of carbohydrate digestion, and its activity determines how quickly and how much glucose is produced from food. The more active the α-amylase enzyme, the faster the conversion of carbohydrates into glucose, the higher the possibility for postprandial blood glucose spikes. Therefore, inhibiting this enzyme's activity is crucial to help control blood glucose levels, particularly after meals in patients with type II diabetes mellitus (Wahyuntari, 2011).

4. Conclusion

The herbarium specimen and 70% ethanol extract of coleus (*S. scutellarioides* (L.) Codd) contain secondary metabolites such as flavonoids, tannins, saponins, and steroids. The 70% ethanol extract of coleus leaves exhibits α-amylase enzyme inhibition activity with an inhibition percentage of 61.03%, although its potential is lower than acarbose as the positive control, which has an enzyme inhibition percentage of 92.16%.

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References

Azwanida, N.N. (2015). A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Medicinal & Aromatic Plants*, *4*(3), 1-6. https://doi.org/10.4172/2167-0412.1000196

Depkes. (2000). *Parameter Standar Umum Ekstrak Tumbuhan Obat*. Jakarta: Departemen Kesehatan Republik Indonesia.

Farnsworth, N. R. (1966). Biological and Phytochemical Screening of Plants. *Journal of Pharmaceutical Sciences*, *55*(3), 225–276. https://doi.org/10.1002/JPS.2600550302

Fathiyah, A. (2023). *Uji Aktivitas Penghambatan Enzim α-Amilase Ekstrak Etanol 70% Rimpang Lempuyang Gajah (Zingiber zerumbet L.) Secara In Vitro*. Skripsi. Jurusan Farmasi Fakultas Kedokteran dan Ilmu Kesehatan UIN Alauddin Makassar.

Grover, J. K., Yadav, S., & Vats, V. (2002). Medicinal Plants of India With Anti-Diabetic Potential. *Journal of Ethnopharmacology*, *81*(1), 81–100. https://doi.org/10.1016/S0378-8741(02)00059-4

Hidayah, N., Jayak Pratama, K., & Raharjo, D. (2023). Aktivitas Penghambatan Enzim Alfa-Amilase Ekstrak Dan Fraksidaun Nipah (*Nypa Fruticans Wurmb*). *Jurnal Ilmiah Wahana Pendidikan, Desember*, *9*(25), 677–684. https://doi.org/10.5281/zenodo.10433927

Kemenkes RI. (2017). *Farmakope Herbal Indonesia Edisi Ii*. Jakarta: Kementerian Kesehatan Republik Indonesia.

Kemenkes RI. (2020). *Pedoman Nasional Pelayanan Kedokteran Tata Laksana Diabetes Melitus Tipe 2 Dewasa*. Jakarta: Kementerian Kesehatan Republik Indonesia.

Lestari, S. (2022). *Uji Aktivitas Antidiabetes Ekstrak Daun Miana (Coleus atropurpureus L.) Pada Mencit Jantan Hiperglikemia Yang Diinduksi Aloksan*. Skripsi. Program Studi Biologi Fakultas Pendidikan Matematika Dan Ilmu Pengetahuan Alam Universitas Pendidikan Indonesia

Lisdawati, V., Mutiatikum, D., Alegantina, S., & N, Y. A. (2012). Karakteristik Daun Miana (*Plectranthus scutellarioides* (L.) Bth.) dan Buah Sirih (*Piper betle* L.) Secara Fisiko Kimia Dari Ramuan Lokal Antimalaria Daerah Sulawesi Utara. *Media Penelitian Dan Pengembangan Kesehatan*, *18*(4), 213–225.

Nafiu, M. O., & Tom Ashafa, A. O. (2017). Antioxidant and Inhibitory Effects of Saponin Extracts from *Dianthus basuticus* Burtt Davy on Key Enzymes Implicated in Type 2 Diabetes In Vitro. *Pharmacognosy Magazine*, *13*(52), 576-582. https://doi.org/10.4103/PM.PM_583_16

Natasya, D., Oktavioni, M., Yusnaidar, Y., & Tarigan, I. L. (2023). Review Senyawa Bioaktif dan Konstituen Kimia Dari Tanaman Coleus Sebagai Kandidat Obat. *Indonesian Journal of Pure and Applied Chemistry*, *6*(2), 61-76. https://doi.org/10.26418/INDONESIAN.V6I2.65149

P, S., Zinjarde, S. S., Bhargava, S. Y., & Kumar, A. R. (2011). Potent α-Amylase Inhibitory Activity of Indian Ayurvedic Medicinal Plants. *BMC Complementary and Alternative Medicine*, *11*(1), 1–10. https://doi.org/10.1186/1472-6882-11-5

Proença, C., Freitas, M., Ribeiro, D., Tomé, S. M., Oliveira, E. F. T., Viegas, M. F., Araújo, A. N., Ramos, M. J., Silva, A. M. S., Fernandes, P. A., & Fernandes, E. (2019). Evaluation of A Flavonoids Library For Inhibition of Pancreatic A-Amylase Towards A Structure–Activity Relationship. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *34*(1), 577–588. https://doi.org/10.1080/14756366.2018.1558221,

Riwanti, P., Izazih, F., & Amaliyah, A. (2020). Pengaruh Perbedaan Konsentrasi Etanol pada Kadar Flavonoid Total Ekstrak Etanol 50,70 dan 96% *Sargassum polycystum* dari Madura. *Journal of Pharmaceutical Care Anwar Medika (J-PhAM)*, *2*(2), 82–95. https://doi.org/10.36932/JPCAM.V2I2.1

Susilawati, Y., Muhtadi, A., Moektiwardoyo, M., & Arifin, P. C. (2016). Aktivitas Antidiabetes Ekstrak Etanol Daun Iler (*Plectranthus scutellarioides* (L.) R.Br.) Pada Tikus Putih Galur Wistar Dengan Metode Induksi Aloksan. *Farmaka*, *14*(2), 82–96. https://doi.org/10.24198/JF.V14I2.9296

Tundis, R., Loizzo, M. R., & Menichini, F. (2010). Natural Products as α-Amylase and α-Glucosidase Inhibitors and their Hypoglycaemic Potential in the Treatment of Diabetes: An Update. *Mini-Reviews in Medicinal Chemistry*, *10*(4), 315–331. https://doi.org/10.2174/138955710791331007

Verpoorte, R., Van der Heijden, R., & Memelink, J. (2000). Engineering The Plant Cell Factory For Secondary Metabolite Production. *Transgenic Research*, *9*(4–5), 323–343. https://doi.org/10.1023/A:1008966404981

Wahyuntari, B. (2011). Penghambat α Amylase*:* Jenis, Sumber, dan Potensi Pemanfaatannya Dalam Kesehatan. *Jurnal Teknologi dan Industri Pangan,* 22(2), 197–201.

Wakhidah, A. Z., & Silalahi, M. (2018). Etnofarmakologi Tumbuhan Miana (*Coleus scutellariodes* (L.) Benth) Pada Masyarakat Halmahera Barat, Maluku Utara. *Jurnal Pro-Life*, *5*(1), 567–578.

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A review of calophyllolide from *Calophyllum inophyllum* L.: isolation, quantification, analytical method, and burn wound healing potential

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Abstract

Background: Calophyllolide is a dipyranocoumarin compound found in *Calophyllum inophyllum* L., known for its antimicrobial and anti-inflammatory properties, which are beneficial for burn wound healing. However, variability in its content and lack of standardized methods remain challenges.

Objective: This review aims to present a literature study on calophyllolide, including its sources, isolation techniques, bioactive content optimization, analytical methods, and pharmacological potential in burn wound healing.

Method: Data were retrieved from Scopus and PubMed using predefined keywords. Articles published in English between 2001 and 2021 and classified as original research were selected. Relevant studies were assessed for quality using the SYRCLE tool (animal studies) and the Young & Solomon checklist (non-clinical research).

Results: Seeds harvested in September had the highest calophyllolide content (0.23%). Enhancement through tissue culture using 2 mg/L IBA yielded up to 45.23 mg/100 g callus. Among analytical techniques, a validated GC-MS method showed high precision and recovery. Pharmacological studies confirmed its activity against *Staphylococcus aureus* and its ability to modulate inflammatory responses.

Conclusion: Calophyllolide shows strong potential as a natural agent for burn wound therapy. Standardized extraction, quantification, and production approaches are essential for further development.

Keywords: Calophyllolide, *Calophyllum inophyllum*, isolation, quantification, burn wound healing

1. Introduction

Calophyllum inophyllum L., commonly known as nyamplung in Indonesia or tamanu in various Pacific regions, is a tropical tree species widely distributed along coastal areas, including in Indonesia (Yuniastuti *et al*., 2021). Traditionally, the oil extracted from its seeds has been utilized as a renewable source for biodiesel production (Adenuga *et al*., 2021). This application continues to be explored and supported by institutions such as the Forestry Research and Development Agency (FORDA), Indonesia (Ong *et al*., 2011). Beyond its industrial relevance, *C. inophyllum* seed oil has long been employed in traditional medicine, especially in Vietnam, for the treatment of burns, skin disorders, rheumatism, and insomnia (Nguyen *et al*., 2017).

Phytochemical investigations of *C. inophyllum* have revealed the presence of a wide spectrum of bioactive secondary metabolites, including coumarins, xanthones, flavonoids, steroids, and triterpenoids (Praveena, 2013; Tsai *et al*., 2012). Among the major constituents identified, calophyllolide has garnered significant attention due to its pharmacological activities, including antimicrobial, cytotoxic, osteogenic, and anti-inflammatory effects

(Itoigawa *et al*., 2001; Yimdjo *et al*., 2004). Other notable compounds isolated from *C. inophyllum* oil include Inophyllum P, Inophyllum B, Calanolide A and B, 12-Oxocalanolide (Kostova & Mojzis, 2007), Inophyllum A, C, D, and E (Itoigawa et al., 2001; Yimdjo *et al*., 2004), Calanolide Gut 70 (Jin *et al*., 2011), and Pseudocalanolide D (Ishikawa, 2000). More recently, two novel neoflavonoids, Tamanolide E1 and E2, were also identified in this oil (Ginigini *et al*., 2019), reflecting the ongoing chemical diversity discovery from this species.

Among these bioactive compounds, calophyllolide stands out due to its dual antimicrobial and anti-inflammatory properties (Gunawan *et al*., 2020), which are particularly relevant in wound healing, especially for burn injuries. Effective burn treatment often requires both infection control and inflammation modulation (Radzikowska-Büchner *et al*., 2023). Thus, calophyllolide presents a promising candidate for therapeutic development in this area.

Despite its promising pharmacological properties, the broader application of calophyllolide remains constrained by several scientific challenges. Its concentration in *C. inophyllum* is influenced by factors such as the specific plant part used, harvesting period, and extraction method (Gupta & Gupta, 2020; Hapsari *et al*., 2023), leading to variability in yield and difficulties in standardization. Additionally, efforts to increase calophyllolide content, through approaches like plant tissue culture, phytohormone induction, and medium modification, are still scattered and lack a unified optimization strategy. While HPLC and GC-MS have been employed for quantification (Jaikumar *et al*., 2017; Liu *et al*., 2015), few studies have provided fully validated analytical methods that ensure accuracy, sensitivity, and reproducibility across different contexts.

This review aims to synthesize and evaluate scientific evidence of calophyllolide, including its sources, isolation techniques, content optimization strategies, analytical methods, and pharmacological potential in burn wound healing. By consolidating existing findings into a coherent narrative, this review seeks to support future research directions and encourage the development of calophyllolide as a therapeutically relevant natural compound.

2. Method

2.1. Literature search strategy

The literature search was conducted in December 2021 using two electronic databases: Scopus and PubMed. The selection of search terms was guided by the main research questions addressed in this review. The following keywords and keyword combinations were used:

"Calophyllolide content", "Calophyllolide AND Calophyllum", "4-substituted coumarin AND Calophyllum", "Calophyllolide expression", "Calophyllolide production", "dipyranocoumarin expression AND Calophyllum", "dipyranocoumarin production AND Calophyllum", "Calophyllolide analysis", "Calophyllolide determination", "antimicrobial AND Calophyllolide", and "anti-inflammatory AND Calophyllolide". Boolean operators (AND, OR) were applied to refine and broaden the search results where necessary.

2.2. Inclusion and exclusion criteria

Articles were included based on the following eligibility criteria: a) indexed in Scopus and/or PubMed, b) written in English, c) categorized as original research articles, and d) published between 2001-2021. Two reviewers independently performed screening and assessed titles and abstracts for relevance to the review objectives. Articles that met the initial criteria were then evaluated in full-text format.

2.2. Quality assessment

Quality assessment of the included studies was conducted with consideration of the study design to ensure methodological rigor and minimize potential bias. *In vitro* studies involving animal models were evaluated using the SYRCLE's Risk of Bias (RoB) tool, which was specifically developed by the Systematic Review Centre for Laboratory Animal Experimentation to assess the internal validity of preclinical animal research (Hooijmans *et al*., 2014). The Young and Solomon critical appraisal checklist was used for non-clinical or basic laboratory research studies. This tool provides structured criteria to assess scientific soundness, including clarity of objectives, appropriateness of methodology, quality of data presentation, and interpretation of findings (Young & Solomon, 2009).

3. Results and discussion

3.1. Article selection process

The initial search using predetermined keywords retrieved a total of 35 scientific articles. After applying the inclusion criteria, 21 articles remained. These articles were further screened based on their title and abstract relevance to the research questions, yielding 14 eligible studies. A full-text screening resulted in 10 final articles being included in this review. One article Nguyen *et al*. (2017) was categorized as an *in vitro* animal study and assessed using

SYRCLE's Risk of Bias tool. The remaining nine articles, classified as basic research, were appraised using the Young and Solomon checklist. The selection process is summarized in **Figure 1**.

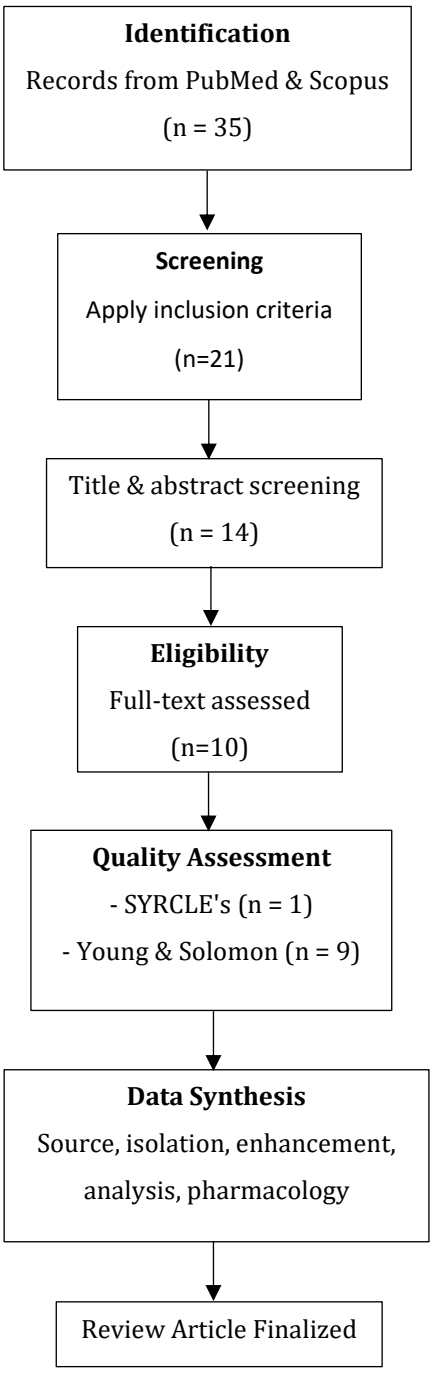

Figure 1. Article selection flowchart

3.2. Natural sources and isolation methods of calophyllolide

Calophyllolide is a naturally occurring coumarin derivative classified under the 4-phenyl pyranocoumarins, with the chemical name 5-methoxy-2,2-dimethyl-6-(2-methylbut-2-enoyl)-10-phenylpyrano [2,3-f] chromen-8(2H)-one (Kalyanaraman *et al*., 2014). Its structure is shown in **Figure 2**.

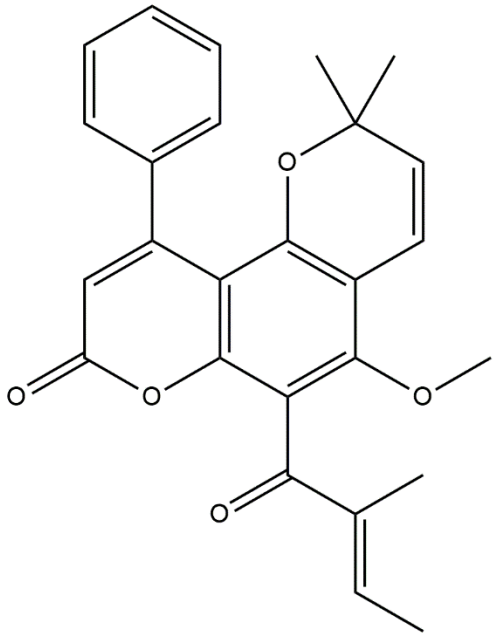

Figure 2. *Calophyllolide* chemical structure

As a member of the phenolic secondary metabolites, coumarins typically possess a C6-C3 core structure (Vermerris & Nicholson, 2008). Phytochemical screening of *C. inophyllum* leaf methanolic extract using cold percolation (2 x 72 hours) revealed the presence of various bioactive compounds such as alkaloids (11.51%), tannins (7.68%), polyphenols (2.53%), triterpenoids (2.48%), flavonoids (2.37%), and saponins (2.16%) (Susanto *et al*., 2017). Calophyllolide has been identified in various parts of the *Calophyllum* genus, with markedly variable content depending on plant part, extraction solvent, and processing method, as detailed in **Table 1**.

Among various plant organs, seeds consistently yielded the highest concentrations of calophyllolide, suggesting they are the primary storage site for this metabolite. For example, Yimdjo *et al*. (2004) obtained 0.118% calophyllolide from *C. inophyllum* seed powder using dichloromethane: methanol (1:1) maceration followed by extensive silica gel chromatography and methanol recrystallization, while Ito *et al*. (2003) reported a much lower yield of only

0.001% from the bark of *C. brasiliense* using acetone extraction at room temperature, followed by silica gel column chromatography and preparative thin-layer chromatography.

Table 1. Comparative calophyllolide contents from different *Calophyllum* plant parts and extraction methods

Plant source	Extraction and isolation method	Calophyllolide content	Reference
Bark of *C. brasiliense*	Room temperature extraction with acetone, silica gel column, and preparative TLC	0.001%	Ito *et al*., 2003
Dried seeds of *C. inophyllum*	CH2Cl2: MeOH (1:1) extraction; silica gel column chromatography with n-hexane: EtOAc	0.118%	Yimdjo *et al.*, 2004
Leaves from the French Polynesian islands	Ethyl acetate extraction (1 week); analyzed with HPLC-UV-DAD	0.0000116% (average)	Laure *et al.*, 2008
Resin of *C. inophyllum* seed oil	Methanol: water (9:1) extraction; vacuum liquid chromatography on silica gel using n-hexane: EtOAc	0.02803% of resin mass	Hien *et al*., 2011
Dried seeds (harvested in September)	Ethanol extraction, silica gel column, and Sephadex LH-20 chromatography	0.23%	Liu *et al.*, 2015
Dried seeds (harvested in December)	Ethanol extraction, silica gel column, and Sephadex LH-20 chromatography	0.16%	Liu *et al.*, 2015
Bark of *C. inophyllum*	Ethanol extraction, silica gel column, and Sephadex LH-20 chromatography	Not detected by HPLC	Liu *et al.*, 2015
Seed shell of *C. inophyllum*	Ethanol extraction, silica gel column, and Sephadex LH-20 chromatography	Not detected by HPLC	Liu *et al.*, 2015
Dried seeds of *C. inophyllum*	Cold press extraction; analyzed with GC-MS	0.0196% of dry seed weight	Charinrat *et al*., 2021

Extraction technique and solvent system also significantly impact calophyllolide yield. Ethanol-based extractions followed by multi-step purification tend to be more efficient. The highest yield (0.23%) was reported by Liu *et al*. (2015) using ethanol extraction, liquid-liquid partitioning with water and ethyl acetate, and subsequent purification with silica gel and Sephadex LH-20 chromatography. Notably, this yield was obtained from seeds harvested in September, whereas a reduced concentration (0.16%) was found in those harvested in December, indicating that seasonal and maturity factors may influence biosynthetic activity and metabolite accumulation.

In contrast, simpler extraction methods or non-seed plant parts tend to yield substantially lower concentrations. For instance, Laure *et al*. (2008) detected only trace amounts of calophyllolide (mean: 0.0000116%) from dried leaf extracts using ethyl acetate maceration over one week. Similarly, no detectable calophyllolide was found in the bark or seed coat using Liu *et al*. (2015) optimized method, reinforcing the idea that calophyllolide is either absent or present in negligible quantities outside the seeds.

Further analysis of processed products like oils also reflects this trend. Using GC-MS, Saechan *et al.* (2021) quantified calophyllolide in cold-pressed oil from oven-dried seeds at 0.0196%. In comparison, (Hien *et al*. 2011) obtained a slightly higher yield of 0.02803% from resinous seed oil extracted with methanol:water (9:1) and purified via vacuum liquid chromatography. These data emphasize that even within seed-derived products, extraction solvents and post-harvest treatments significantly affect compound recovery.

3.3. Optimization of calophyllolide content

Despite its pharmacological potential, the natural abundance of calophyllolide remains extremely low, making its large-scale extraction inefficient and unsustainable. This limitation has driven interest in biotechnological approaches, particularly plant tissue culture, as a means to enhance calophyllolide production. Tissue culture provides a controlled environment for inducing and optimizing secondary metabolite synthesis (Chandran *et al.*, 2020), thereby offering a promising platform for consistent and scalable production.

Pawar *et al*. (2007) investigated the effect of different types and concentrations of phytohormones on calophyllolide accumulation in *C. inophyllum* callus cultures derived from various explants (seeds, nodal/internodal segments, and leaves). The highest calophyllolide content (45.23 mg/100 g callus) was observed in seed-derived callus treated with 2 mg/L indole-3-butyric acid (IBA). Lower yields were recorded in nodal/internodal and leaf callus cultures under different hormone combinations. In contrast, lower yields were observed in calli from nodal/internodal, and leaf callus cultures, suggesting that callus cultures origin and hormonal balance critically influence biosynthetic capacity.

Further research by Pawar & Thengane (2009) investigated suspension cultures and found that the application of IBA (4.90 μM), alone or in combination with BAP or Picloram, significantly boosted calophyllolide synthesis. This was further optimized by modifying nitrate,

sulfate, and vitamin concentrations in the culture medium, resulting in an impressive 85-fold increase in dipyranocoumarin content compared to baseline levels.

To further augment metabolite levels, Pawar & Thengane (2011) introduced abiotic elicitors into the culture system. Among the tested compounds, cadmium, copper, chromium, and calcium chloride, cadmium produced the most pronounced enhancement in calophyllolide content. This suggests that stress-response pathways activated by metal ions may play a key role in stimulating coumarin biosynthesis. However, caution is warranted due to potential toxicity and regulatory limitations associated with heavy metal use in bioproduction systems.

While tissue culture has demonstrated considerable success in enhancing calophyllolide yield, its scalability and cost-effectiveness remain points of concern for industrial application. Therefore, future research should aim to integrate genetic engineering, elicitor screening, and environmental manipulation in field-grown plants (Malu *et al*., 2025) to establish a more holistic and sustainable strategy for calophyllolide production.

3.4. Analytical methods for calophyllolide quantification

As interest in the pharmacological application of calophyllolide continues to grow, it is essential to develop accurate, sensitive, and reproducible analytical methods for its quantification. Several studies have investigated various instrumental techniques, particularly high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), to determine the content of calophyllolide in *C. inophyllum*.

Liu *et al*. (2015) employed an HPLC method using a Cosmosil 5C18-AR-II analytical column coupled with a μBondpack C18 pre-column. The separation was achieved through gradient elution with a water: acetonitrile mixture. The elution profile consisted of water: acetonitrile (30:70, v/v) from 0 to 20 minutes, followed by a shift to 100% acetonitrile from 20 to 40 minutes. Detection was carried out at 254 nm using a UV detector. While the method demonstrated effective separation of calophyllolide, it lacked critical validation data such as linearity, precision, and recovery, thereby limiting its applicability for quality control or regulatory purposes.

A similar limitation was observed in the study by Laure *et al*. (2008), who utilized HPLC with UV-DAD detection to analyze calophyllolide in *C. inophyllum* leaf extracts. The method involved a gradient of isopropanol: isooctane (1–20%, v/v) over 25 minutes, followed by a 15-minute stabilization period. Detection was performed at 360 nm. However, like the method by

Liu *et al*. (2015), this protocol remained largely descriptive and did not report analytical validation parameters, leaving uncertainty regarding its reproducibility, specificity, or robustness.

In contrast, Hien *et al*. (2011) developed and validated a GC-MS method for calophyllolide quantification. Various column temperature programs were evaluated, with optimal conditions identified as an initial column temperature of 80 °C, followed by a temperature ramp of 40 °C/min to 300 °C, maintained for 9 minutes. Under these parameters, calophyllolide exhibited a sharp retention time of 12.83 ± 0.006 minutes with preserved ion characteristics. The method demonstrated a linear response within the 3.125–50 µg/mL range, intra- and inter-day precision with relative standard deviation (RSD) below 3%, and an average recovery rate of 101.22 ± 1.98% (RSD = 1.95%). This comprehensive validation sets the GC-MS method apart as the most reliable analytical protocol currently available for calophyllolide quantification. Its high precision and recovery rates support its use not only in research settings but also in routine quality control workflows for *C. inophyllum*-based preparations.

Overall, while HPLC-based methods provide a foundation for calophyllolide analysis, future work should prioritize full method validation to meet international standards. The established GC-MS method may serve as a benchmark for future protocol development, or even as a standardized analytical method for regulatory and industrial applications.

3.5. Burn wound healing potential of calophyllolide

Calophyllolide, a bioactive coumarin isolated from *C. inophyllum*, has garnered increasing attention due to its antimicrobial and anti-inflammatory properties (Gunawan *et al*., 2020). These two pharmacological actions are particularly crucial in addressing key challenges associated with burn wound healing, which involves complex inflammatory responses, a high risk of infection, and delayed tissue regeneration (Su *et al*., 2024).

Burn injuries disrupt the skin's protective barrier, creating a nutrient-rich environment that fosters microbial colonization. Under these compromised conditions, opportunistic pathogens, notably *Staphylococcus aureus* and *Pseudomonas aeruginosa*, can proliferate rapidly. This colonization often leads to delayed re-epithelialization, prolonged inflammation, and increased risk of systemic infection or sepsis (Hall et al., 2018). Consequently, effective burn wound management requires agents capable of both combating infection and modulating inflammation.

Over the years, several antimicrobial agents have been clinically employed, including silver nitrate, silver sulfadiazine, bacitracin, and neomycin (Glasser *et al*., 2010), as well as more advanced materials such as silver-chitosan acetate nanocomposites (Huang *et al.*, 2011). Likewise, anti-inflammatory treatments such as ibuprofen (Ambler *et al*., 2005) and the topical anti-IL-6R antibody MR16-1 (Sakimoto *et al*., 2012) have been shown to support tissue regeneration and reduce inflammatory damage. In this context, calophyllolide presents a unique advantage as a single compound that exhibits both antimicrobial and anti-inflammatory activity, suggesting its potential as a dual-function agent for burn wound treatment.

Yimdjo *et al*. (2004) demonstrated that calophyllolide exhibited the strongest antibacterial activity among various compounds isolated from *C. inophyllum*, including caloxanthone A, calophynic acid, brasiliensic acid, and several inophyllum derivatives. When tested against *S. aureus* at a concentration of 20 μg per disk, calophyllolide produced an inhibition zone diameter of 16.0 mm, greater than that observed for other individual compounds and crude extracts of the root bark and seeds.

In addition to its antimicrobial effect, calophyllolide has garnered attention for its anti-inflammatory properties, which could offer significant therapeutic value in managing complex wound healing scenarios. A study by Nguyen *et al*. (2017) explored this aspect using a murine full-thickness incision model, comparing the efficacy of calophyllolide (12 mg/mL), Povidone Iodine (PI, 200 mg/mL), and Phosphate Buffered Saline (PBS) over a 14-day treatment period. While the data are derived from a single preclinical source, the depth of investigation into inflammatory modulation warrants closer examination.

One of Nguyen *et al*. (2017) most notable findings was the phenotypic polarization of macrophages following calophyllolide treatment. There was a significant downregulation of pro-inflammatory M1 markers CD14 and CD127, alongside upregulation of reparative M2 markers CD163 and CD206. This polarization suggests a shift in the local immune environment toward resolution and regeneration, aligning with the physiological transition from the inflammatory to the proliferative phase of wound healing (Krzyszczyk *et al*., 2018).

Moreover, calophyllolide administration markedly suppressed pro-inflammatory cytokines, IL-1β, IL-6, and TNF-α, by up to 90% at days 5 and 7 post-injury (Nguyen *et al*., 2017). This was accompanied by a transient elevation of IL-10, a cytokine closely associated with anti-inflammatory macrophage activity and tissue repair (Kessler *et al*., 2017). Although the IL-10

surge diminished by day 7, its early presence may be critical for dampening excessive inflammation and initiating resolution pathways.

Another key outcome from the same study was the suppression of myeloperoxidase (MPO) activity, a surrogate marker for neutrophil infiltration and oxidative stress (Khan *et al*., 2018). Calophyllolide's ability to reduce MPO by approximately fourfold relative to PI suggests a protective mechanism against neutrophil-mediated tissue injury (Nguyen *et al*., 2017). While the study was limited to an animal model, and the findings require validation in human tissues, the comprehensive dataset provides a compelling case for further translational research.

The studies in this review span a wide range of research exploring calophyllolide, from its natural sources, extraction methods, and bioactive content optimization strategies to its pharmacological potential, particularly in wound healing. The strength of this review lies in its integrated discussion of both phytochemical and biomedical aspects, providing a comprehensive foundation for further development of calophyllolide as a therapeutic candidate. Nonetheless, the review is confined to studies published in English and sourced from selected databases, which may exclude relevant findings published in other languages or outside major indexing platforms. Such restrictions may limit the breadth of perspectives captured and underscore the need for broader, multi-source evidence synthesis in future reviews

4. Conclusion

This review highlights that *Calophyllum inophyllum* seeds, particularly those harvested in September, are the richest natural source of calophyllolide. Optimization through plant tissue culture, especially with 2 mg/L IBA, has shown promising results in increasing its content. Among analytical methods, the validated GC-MS approach provides reliable quantification. With its dual antimicrobial and anti-inflammatory properties, calophyllolide is a strong candidate for burn wound therapy. These findings support further development of calophyllolide-based products and reinforce its potential contribution to natural compound-based pharmaceuticals.

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References

Adenuga, A. A., Oyekunle, J. A. O., & Idowu, O. O. (2021). Pathway To Reduce Free Fatty Acid Formation In *Calophyllum inophyllum kernel* Oil: A Renewable Feedstock For Biodiesel Production. *Journal of Cleaner Production*, *316*(2021), 1-10. https://doi.org/10.1016/j.jclepro.2021.128222

Ambler, J. J. S., Zideman, D. A., & Deakin, C. D. (2005). The Effect of Topical Non-Steroidal Anti-Inflammatory Cream On The Incidence and Severity of Cutaneous Burns Following External DC Cardioversion. *Resuscitation*, *65*(2), 173–178. https://doi.org/10.1016/j.resuscitation.2004.11.013

Chandran, H., Meena, M., Barupal, T., & Sharma, K. (2020). Plant Tissue Culture As A Perpetual Source For Production of Industrially Important Bioactive Compounds. *Biotechnology Reports*, *26*(2020), 1-10. https://doi.org/10.1016/j.btre.2020.e00450

Ginigini, J., Lecellier, G. J., Nicolas, M., Nour, M., Hnawia, E., Lebouvier, N., Herbette, G., Lockhart, P., & Raharivelomanana, P. (2019). Chemodiversity of *Calophyllum inophyllum* L. Oil Bioactive Components Related To Their Specific Geographical Distribution In The South Pacific Region. *PeerJ*, *2019*(5). https://doi.org/10.7717/peerj.6896

Glasser, J. S., Guymon, C. H., Mende, K., Wolf, S. E., Hospenthal, D. R., & Murray, C. K. (2010). Activity of Topical Antimicrobial Agents Against Multidrug-Resistant Bacteria Recovered From Burn Patients. *Burns*, *36*(8), 1172–1184. https://doi.org/10.1016/j.burns.2010.05.013

Gunawan, S., Pamungkas, B., Primaswari, C. S., Hapsari, S., & Aparamarta, H. W. (2020). Calophyllolide Separation from *Calophyllum inophyllum* Oil by Silica Gel Adsorption. *Materials Science Forum*, *988*(12), 101–107. https://doi.org/10.4028/www.scientific.net/MSF.988.101

Gupta, S., & Gupta, P. (2020). The Genus *Calophyllum*: Review of Ethnomedicinal Uses, Phytochemistry and Pharmacology. In *Bioactive Natural products in Drug Discovery* (pp. 215–242). Springer Singapore. https://doi.org/10.1007/978-981-15-1394-7_5

Hall, C., Hardin, C., Corkins, C.J., Jiwani, A.Z., Fletcher, J., Carlsson, A., Chan, R. (2018). Pathophysiologic mechanisms and current treatments for cutaneous sequelae of burn wounds. Compr. Physiol. 8, 371–405. https://doi.org/10.1002/cphy.c170016

Hapsari, S., Jadid, N., Aparamarta, H. W., & Gunawan, S. (2023). Impact of Solvent Type, Solvent-Water Concentration, and Number of Stages On The Extraction of Coumarin Mixture From Tamanu (*Calophyllum inophyllum*) Oil and Its Antioxidant Activity. *Arabian Journal of Chemistry*, *16*(2), 1-11. https://doi.org/10.1016/j.arabjc.2022.104449

Hien, H. M., Heng, P. W. S., van Thi, N., Quynh Cu, N. K., & Hue, V. T. B. (2011). Development and Validation of A GC-MS Method For Rapid Determination of Calophyllolide in *Calophyllum inophyllum* L.: A Quality Control Approach. *Chinese Journal of Natural Medicines*, *9*(6), 429–434. https://doi.org/10.3724/SP.J.1009.2011.00429

Hooijmans, C. R., Rovers, M. M., De Vries, R. B. M., Leenaars, M., Ritskes-Hoitinga, M., & Langendam, M. W. (2014). SYRCLE's Risk of Bias Tool For Animal Studies. *BMC Medical Research Methodology*, *14*(1), 1–9. https://doi.org/10.1186/1471-2288-14-43

Huang, L., Dai, T., Xuan, Y., Tegos, G. P., & Hamblin, M. R. (2011). Synergistic Combination of Chitosan Acetate With Nanoparticle Silver As A Topical Antimicrobial: Efficacy Against Bacterial Burn Infections. *Antimicrobial Agents and Chemotherapy*, *55*(7), 3432–3438. https://doi.org/10.1128/AAC.01803-10

Ishikawa, T. (2000). Anti HIV-1 Active Calophyllum Coumarins: Distribution, Chemistry, and Activity. *HETEROCYCLES*, *53*(2), 453-474. https://doi.org/10.3987/REV-99-526

Ito, C., Itoigawa, M., Mishina, Y., Cechinel Filho, V., Enjo, F., Tokuda, H., Nishino, H., & Furukawa, H. (2003). Chemical Constituents of *Calophyllum brasiliense*. 2. Structure of Three New Coumarins and Cancer Chemopreventive Activity Of 4-Substituted Coumarins. *Journal of Natural Products*, *66*(3), 368–371. https://doi.org/10.1021/np0203640

Itoigawa, M., Ito, C., Tan, H. T.-W., Kuchide, M., Tokuda, H., Nishino, H., & Furukawa, H. (2001). Cancer Chemopreventive Agents, 4-Phenylcoumarins from *Calophyllum inophyllum*. *Cancer Letters*, *169*(1), 15–19. https://doi.org/10.1016/S0304-3835(01)00521-3

Jaikumar, K., Sheik, N. M., John Wyson, W., Deventhiran, M., Babu, A., Anand, D., & Saravanan, P. (2017). In Silico Docking Analysis of Bioactive Compounds From *Calophyllum inophyllum* L. Ethanol Leaf Extract Against Egfr Protein. *Asian Journal of Pharmaceutical and Clinical Research*, *10*(8), 214-219. https://doi.org/10.22159/ajpcr.2017.v10i8.18972

Jin, L., Tabe, Y., Kimura, S., Zhou, Y., Kuroda, J., Asou, H., Inaba, T., Konopleva, M., Andreeff, M., & Miida, T. (2011). Antiproliferative and Proapoptotic Activity of GUT-70 Mediated Through Potent Inhibition of Hsp90 In Mantle Cell Lymphoma. *British Journal of Cancer*, *104*(1), 91–100. https://doi.org/10.1038/sj.bjc.6606007

Kalyanaraman, L., Sree Ganesh, K. K., Kumar, R. M., Pichai, R., & Vyas, K. (2014). Characterization of A Bioactive Derivative of Calophyllolide By 2d NMR and LC-MS/MS. *Journal of Liquid Chromatography & Related Technologies*, *37*(8), 1087–1093. https://doi.org/10.1080/10826076.2013.765463

Kessler, B., Rinchai, D., Kewcharoenwong, C., Nithichanon, A., Biggart, R., Hawrylowicz, C. M., Bancroft, G. J., & Lertmemongkolchai, G. (2017). Interleukin 10 Inhibits Pro-Inflammatory Cytokine Responses and Killing of *Burkholderia pseudomallei*. *Scientific Reports*, *7*(1), 1-11. https://doi.org/10.1038/srep42791

Khan, A. A., Alsahli, M. A., & Rahmani, A. H. (2018). Myeloperoxidase as an Active Disease Biomarker: Recent Biochemical and Pathological Perspectives. *Medical Sciences*, *6*(2), 1-21. https://doi.org/10.3390/medsci6020033

Kostova, I., & Mojzis, J. (2007). Biologically Active Coumarins As Inhibitors of HIV-1. *Future HIV Therapy*, *1*(3), 315–329. https://doi.org/10.2217/17469600.1.3.315

Krzyszczyk, P., Schloss, R., Palmer, A., & Berthiaume, F. (2018). The Role of Macrophages in Acute and Chronic Wound Healing and Interventions to Promote Pro-wound Healing Phenotypes. *Frontiers in Physiology*, *9*, 1-22. https://doi.org/10.3389/fphys.2018.00419

Laure, F., Raharivelomanana, P., Butaud, J. F., Bianchini, J. P., & Gaydou, E. M. (2008). Screening of Anti-HIV-1 Inophyllums by HPLC-DAD of *Calophyllum inophyllum* Leaf Extracts From French Polynesia Islands. *Analytica Chimica Acta*, *624*(1), 147–153. https://doi.org/10.1016/j.aca.2008.06.046

Liu, W. H., Liu, Y. W., Chen, Z. F., Chiou, W. F., Tsai, Y. C., & Chen, C. C. (2015). Calophyllolide Content in *Calophyllum inophyllum* At Different Stages of Maturity and Its Osteogenic Activity. *Molecules*, *20*(7), 12314–12327. https://doi.org/10.3390/molecules200712314

Malu, M., Chatterjee, J., Choudhary, D., Ramakrishna, W., & Kumar, R. (2025). Biotic, Abiotic, and Genetic Elicitors As A New Paradigm For Enhancing Alkaloid Production For Pharmaceutical Applications. *South African Journal of Botany*, *177*, 579–597. https://doi.org/10.1016/j.sajb.2024.12.022

Nguyen, V.-L., Truong, C.-T., Nguyen, B. C. Q., Vo, T.-N. Van, Dao, T.-T., Nguyen, V.-D., Trinh, D.-T. T., Huynh, H. K., & Bui, C.-B. (2017). Anti-inflammatory and Wound Healing Activities of Calophyllolide Isolated From *Calophyllum inophyllum* Linn. *PLOS ONE*, *12*(10), 1–16. https://doi.org/10.1371/journal.pone.0185674

Ong, H. C., Mahlia, T. M. I., Masjuki, H. H., & Norhasyima, R. S. (2011). Comparison of Palm Oil, *Jatropha curcas* and *Calophyllum inophyllum* for Biodiesel: A review. *Renewable and Sustainable Energy Reviews*, *15*(8), 3501–3515. https://doi.org/10.1016/j.rser.2011.05.005

Pawar, K. D., Joshi, S. P., Bhide, S. R., & Thengane, S. R. (2007). Pattern of Anti-HIV Dipyranocoumarin Expression In Callus Cultures of *Calophyllum inophyllum* Linn. *Journal of Biotechnology*, *130*(4), 346–353. https://doi.org/10.1016/j.jbiotec.2007.04.024

Pawar, K. D., & Thengane, S. R. (2009). Influence of Hormones and Medium Components On Expression of Dipyranocoumarins In Cell Suspension Cultures of *Calophyllum inophyllum* L. *Process Biochemistry*, *44*(8), 916–922. https://doi.org/10.1016/j.procbio.2009.03.005

Pawar, K. D., & Thengane, S. R. (2011). Influence of Abiotic Elicitation On Production of Dipyranocoumarins In Suspension Cultures of *Calophyllum inophyllum* L. *Acta Physiologiae Plantarum*, *33*, 2149–2158.

Praveena, C. (2013). Phytochemical Investigation of *Calophyllum inophyllum* Linn. *Natural Products Chemistry & Research*, *1*(4), 4–7. https://doi.org/10.4172/2329-6836.1000119

Radzikowska-Büchner, E., Łopuszyńska, I., Flieger, W., Tobiasz, M., Maciejewski, R., & Flieger, J. (2023). An Overview of Recent Developments in the Management of Burn Injuries. *International Journal of Molecular Sciences*, *24*(22), 16357. https://doi.org/10.3390/ijms242216357

Saechan, C., Kaewsrichan, J., Leelakanok, N., & Petchsomrit, A. (2021). Antioxidant in cosmeceutical products containing Calophyllum inophyllum oil. *OCL*, *28*, 28. https://doi.org/10.1051/ocl/2021015

Sakimoto, T., Sugaya, S., Ishimori, A., & Sawa, M. (2012). Anti-inflammatory Effect of IL-6 Receptor Blockade In Corneal Alkali Burn. *Experimental Eye Research*, *97*(1), 98–104. https://doi.org/10.1016/j.exer.2012.02.015

Su, H., Chen, Y., Jing, X., Zhao, X., Sun, H., Liu, Z., Qiu, Y., Zhang, Z., Guan, H., & Meng, L. (2024). Antimicrobial, Antioxidant, and Anti-Inflammatory Nanoplatform for Effective Management of Infected Wounds. *Advanced Healthcare Materials*, *13*(5). https://doi.org/10.1002/adhm.202302868

Susanto, D. F., Aparamarta, H. W., Widjaja, A., & Gunawan, S. (2017). Identification of Phytochemical Compounds in *Calophyllum inophyllum* leaves. *Asian Pacific Journal of Tropical Biomedicine*, *7*(9), 773–781. https://doi.org/10.1016/j.apjtb.2017.08.001

Tsai, S. C., Liang, Y. H., Chiang, J. H., Liu, F. C., Lin, W. H., Chang, S. J., Lin, W. Y., Wu, C. H., & Weng, J. R. (2012). Anti-inflammatory Effects of *Calophyllum inophyllum* L. in RAW264.7 Cells. *Oncology Reports*, *28*(3), 1096–1102. https://doi.org/10.3892/or.2012.1873

Vermerris, W., & Nicholson, R. (2008). Families of Phenolic Compounds and Means of Classification. In *Phenolic Compound Biochemistry* (pp. 1–34). Springer Netherlands. https://doi.org/10.1007/978-1-4020-5164-7_1

Yimdjo, M. C., Azebaze, A. G., Nkengfack, A. E., Meyer, A. M., Bodo, B., & Fomum, Z. T. (2004). Antimicrobial and Cytotoxic Agents From *Calophyllum inophyllum*. *Phytochemistry*, *65*(20), 2789–2795. https://doi.org/10.1016/j.phytochem.2004.08.024

Young, J. M., & Solomon, M. J. (2009). How To Critically Appraise An Article. *Nature Clinical Practice Gastroenterology and Hepatology*, *6*(2), 82–91. https://doi.org/10.1038/ncpgasthep1331

Yuniastuti, E., Anjani, I., Nandariyah, & Delfianti, M. N. I. (2021). Morphological Characterization of *Calophyllum inophyllum* As A Biodiesel. *IOP Conference Series: Earth and Environmental Science*, *724*(1), 1-8. https://doi.org/10.1088/1755-1315/724/1/012026