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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. 20 No. 2 of 2024** can be published. This issue contains ten articles, three on the pharmaceutical science topic and seven from the clinical & community pharmacy scope. The article presented in the Clinical and Community Pharmacy scope reviews the effectiveness of therapy for patients in hospitals and the evaluation of pharmacist services in health facilities. Meanwhile, papers in the pharmaceutical science area include reviews and tests of drugs made from natural ingredients and their approach through in vitro and in silico methods.

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, December 2024
Editor in Chief

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Bioinformatics analysis of radix *Angelica dahurica*, *Chuanxiong* rhizoma, and *Cyperi* rhizoma for COVID-19 treatment

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Abstract

Background: The COVID-19 pandemic, which has unfolded over the past years, poses significant threats to global public health and socioeconomic well-being. Three herbs (radix *Angelica dahurica, Chuanxiong* rhizoma, and *Cyperi* rhizoma) have been long utilized in combination for treating common colds and flu.

Objective: To analyze potential biotargets and explore possible effects of plant-derived compounds from 3 herbs towards COVID-19 treatment.

Method: Bioinformatics databases and network pharmacology were employed to identify bioactive compounds and their biotargets with integrated statistical calculation, followed by Gene Ontology enrichment. KEGG pathway analysis was performed to elucidate the involvement of selected bioactive compounds in COVID-19-related processes.

Results: Network pharmacology highlighted essential receptors, cytokines, and signaling proteins. Gene Ontology analysis revealed associations with signal transduction, RNA transcription enzymes, and crucial cellular components. Molecular function analysis emphasized interactions related to virus entry. KEGG analysis uncovered 32 potential targets across various pathways, elucidating their role in inflammation and cytokine storms.

Conclusion: This study provides new insight for the molecular mechanisms underlying the therapeutic potential of a combination of radix *Angelica dahurica, Chuanxiong* rhizoma, and *Cyperi* rhizoma against COVID-19. The identified targets and pathways offer new directions for further experimental validation, paving the way for potential therapeutic interventions for COVID-19.

Keywords: radix *Angelica dahurica, Cyperi* rhizome, *Chuanxiong* rhizome, COVID-19, bioinformatics

1. Introduction

In the context of the COVID-19 pandemic, the urgent need for effective treatments has prompted researchers to explore unconventional yet potentially impactful sources (Filip *et al*., 2022). Amid the COVID-19 pandemic, Traditional Chinese Medicine (TCM) has emerged as a potential therapeutic avenue (Ding *et al*., 2022). In China, TCM has shown efficacy in various conditions, including COVID-19 (Fan *et al*., 2020). TCM herbal formulas, based on specific combinations, have demonstrated effectiveness in alleviating respiratory symptoms and contributing to disease severity reduction (Fan *et al*., 2020). Despite differing perspectives between TCM and Western medicine on COVID-19, the scientific rationale of administration of TCM herbs in patients is evident (Ding *et al*., 2022).

Radix *Angelica dahurica, Chuanxiong* rhizoma, and *Cyperi* rhizoma, prominent constituents of traditional Vietnamese medicinal formulations, such as "Cam Xuyen Huong" (CXH) have long been recognized for their therapeutic efficacy in managing flu-like symptoms. This remedy aligns with traditional medicine principles for wind-cold invasion diseases, sharing symptoms with COVID-19 (Liu, 2010).

Pharmacological studies reveal that main herbal components of CXH possess antiviral, anti-inflammatory, anti-thrombotic, and immune-modulatory effects (Donkor *et al*., 2016), (Safa *et al.*, 2020), (Zhang *et al*., 2019), (Kamala *et al*., 2018), (Lee *et al*., 2020). Rich in essential oils and polyphenols, CXH shows promise as a potential agent for COVID-19 prevention and treatment (Solnier & Fladerer, 2020), (Asif *et al*., 2020). Notably, these traditional remedies have gained widespread acceptance for addressing common respiratory ailments, including colds and flu, with symptoms akin to those observed in most of mild or moderate COVID-19 patients. This raises a compelling question about the intentional or unintentional use of these traditional remedies for COVID-19 patients and underscores the need for a rigorous scientific investigation into their bioactive components and potential efficacy.

By employing advanced bioinformatics analysis and network pharmacology, our research aims to unravel the molecular underpinnings of radix *Angelica dahurica*, *Chuanxiong* rhizoma, and *Cyperi* rhizoma. Through the integration of computational methodologies, we seek to elucidate the potential therapeutic mechanisms of these traditional herbal compounds against COVID-19. This exploration is not only rooted in the rich tapestry of Vietnamese traditional medicine but also aligns with the pressing global imperative to identify safe and effective treatments for COVID-19. The significance of our research lies in bridging traditional wisdom with contemporary scientific methodologies, offering a unique perspective that could influence clinical considerations and public health strategies in the ongoing battle against the COVID-19 pandemic.

2. Method

Study was performed based on the methods previously described (Kang *et al*., 2024, Huang *et al*., 2021, Loganathan *et al*., 2024), with study design flow chart presented in **Figure 1**.

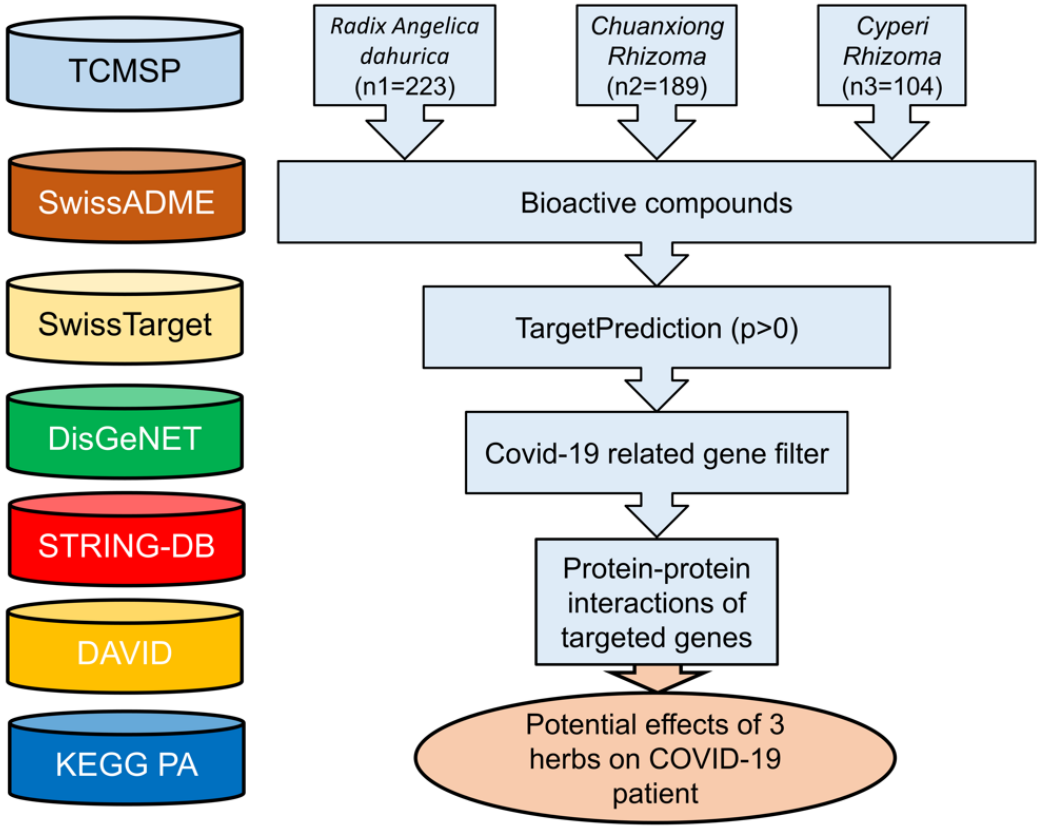

Figure 1: Flowchart of study design

2.1. Retrieval of bioactive compounds

The Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) (Ru *et al*., 2014), available at (https://tcmsp-e.com/tcmsp.php) was employed to retrieve a comprehensive list of bioactive compounds from radix *Angelica dahurica*, *Chuanxiong* rhizoma, and *Cyperi* rhizoma. This initial step aims to identify the chemical constituents present in these herbal sources. List of chemical identities were converted into SMILES (Simplified Molecular Input Line Entry System) as recorded in PUBCHEM database.

2.2. Drug-likeness assessment

SwissADME (Daina *et al.*, 2017) was utilized for assessing the drug-likeness properties of the identified bioactive compounds, using SMILES codes as input. The bioactive compounds were filtered based on their predicted physicochemical properties, following Lipinski's Rule of Five, to ensure their drug-likeness.

2.3. Biotarget prediction

SwissTargetPrediction (Gfeller *et al*., 2014) was employed to predict potential biotargets associated with the selected drug-like compounds. This computational approach helps in identifying the proteins or targets that may interact with the bioactive compounds, facilitating a deeper understanding of their pharmacological mechanisms. Compounds with a probability score greater than 0 in SwissTargetPrediction were retained, indicating a possible interaction with the selected targets. The higher probabilities indicate stronger predicted interactions.

2.4. Selection of target genes and network pharmacology analysis

Web-based DisGeNET tool (Pinero *et al*., 2017) was utilized to filter and prioritize target genes related to COVID-19. Subsequently, a network pharmacology analysis was conducted using STRING-DB web tool (Szklarczyk *et al*., 2021) to construct an interaction network between proteins under effect of bioactive compounds. The most interacting cluster was selected to display with high confidence value set to 0.7. This analysis provides insights into the potential synergistic effects at molecular interactions.

2.5. Functional GO analysis

DAVID bioinformatics tool (Sherman *et al*., 2022) was employed for functional Gene Ontology (GO) analysis. This step aims to categorize and annotate the identified target genes based on their biological processes, molecular functions, and cellular components, elucidating the functional implications of the selected bioactive compounds.

2.6. Pathway analysis

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (Kanehisa & Goto, 2000) was conducted to unravel the biological pathways associated with the identified target genes. This analysis will offer a systematic understanding of how the bioactive compounds from radix *Angelica dahurica*, *Chuanxiong* rhizoma, and *Cyperi* rhizoma may impact various cellular processes and signaling pathways relevant to COVID-19.

3. Result and discussion

3.1. Retrieval list of bioactive compounds and drug-likeness assessment

Using the TCMSP database, we can look up available compounds on the system for radix *Angelica dahurica* (223 compounds), *Chuanxiong* rhizoma (189 compounds), and *Cyperi* rhizoma (104 compounds). The SwissADME web tool was utilized for screening, eliminating compounds

violating Lipinski's rule of 5 (more than one of the criteria) in combination with the analysis of pharmacokinetic parameters (ADME). The screening process, led to the identification of 58 compounds with favorable drug-likeness and oral bioavailability (Data not shown). Notably, these compounds exhibited high permeability across Caco-2 cell membranes, emphasizing their potential for effective absorption and distribution via oral administration. For further screening of target proteins, a short list of bioactive compounds was selected. **Table 1** presented all selected compounds adhere to Lipinski rule and exhibit drug-likeness (DL) values greater than 0.2, along with oral bioavailability (OB) exceeding 20%. Additionally, these compounds display high permeability across Caco-2 cell membranes, all with values above 0.7 (log Papp in 10 cm/s), indicating their potential for effective absorption, distribution via oral administration, and there is a high possibility giving a therapeutic effect.

Table 1. Screening results based on the Lipinski rule and ADME parameters

No.	Compounds	*Caco-2* >0.7	Oral bioavailability %>=20	Drug-likeness >=0,2	Herbs (*)
1	Isoimperatorin	0.97	45.46	0.23	RAD
2	Prangenin	0.8	43.6	0.29	RAD
3	Byakangelicol	0.76	41.42	0.36	RAD
4	Phellopterin	0.98	40.19	0.28	RAD
5	Alloisoimperatorin	0.9	34.8	0.22	RAD
6	Ammidin	1.13	34.55	0.22	RAD
7	Cnidilin	1.03	32.69	0.28	RAD
8	9-[[(2R)-3,3-dimethyloxiran-2-yl]methoxy]furo[3,2-g]chromen-7-one	0.82	29.78	0.29	RAD
9	9-hydroxy-4-(3-methylbut-2-enoxy)furo[3,2-g]chromen-7-one	0.9	28.06	0.25	RAD
10	Oxypeucedanin	0.85	24.9	0.3	RAD
11	Perlolyrine	0.88	65.95	0.27	RLW
12	Senkyunone	1.15	47.66	0.24	RLW
13	Wallichilide	0.82	42.31	0.71	RLW
14	1,4-Epoxy-16-hydroxyheneicos-1,3,12,14,18-pentaene	1.28	45.1	0.24	RC
15	Isodalbergin	0.8	35.45	0.2	RC
16	Rosenonolactone	0.72	79.84	0.37	RC
17	Hyndarin	1	73.94	0.64	RC

(*) Radix *Angelica dahurica* (RAD), *Chuanxiong* rhizoma (RLW), *Cyperi* rhizoma (RC)

3.2. Selection of target genes and network pharmacology analysis

Among the 17 compounds screened, their respective SMILES codes were collected, and a target search was conducted using the online tool SwissTargetPrediction, identified 447 genes with a probability greater than 0. Subsequent comparison and categorization of genes associated with SARS-CoV-2-induced pneumonia were performed utilizing the DisGeNet database toolkit. The screening outcomes revealed 162 genes that overlapped with the initial set of 614 genes targeted by

03 herbs and COVID-19 related genes by database (**Figure 2A**). The list of overlapping genes (162) was used as input for STRING-DB to construct the Protein-Protein interaction network. The result is presented in **Figure 2B**. The network diagram shows the most important interactions with high possibilities between various nodes, including important receptors (**IGF1R, EGFR, INSR, EPOR, AR**, **ESR1, IL2RB**), cytokine (**IL2**), several central signal conductors (**AKT1, MAPK8, JAK1, JAK2, MDM2**).

The protein-protein interaction network analysis revealed critical interactions between receptors, cytokines, and signal transduction proteins, notably **IGF1R, EGFR, and IL2**. The bioactive compounds from radix *Angelica dahurica*, *Chuanxiong* rhizoma, and *Cyperi* rhizoma may collectively enhance the modulation of these targets. For instance, the combination of these herbs could result in synergistic effects on the inhibition of pro-inflammatory cytokines like **IL2**, thereby potentially reducing cytokine storm severity in COVID-19 patients. Further experimental validation is required to confirm these bioinformatic predictions.

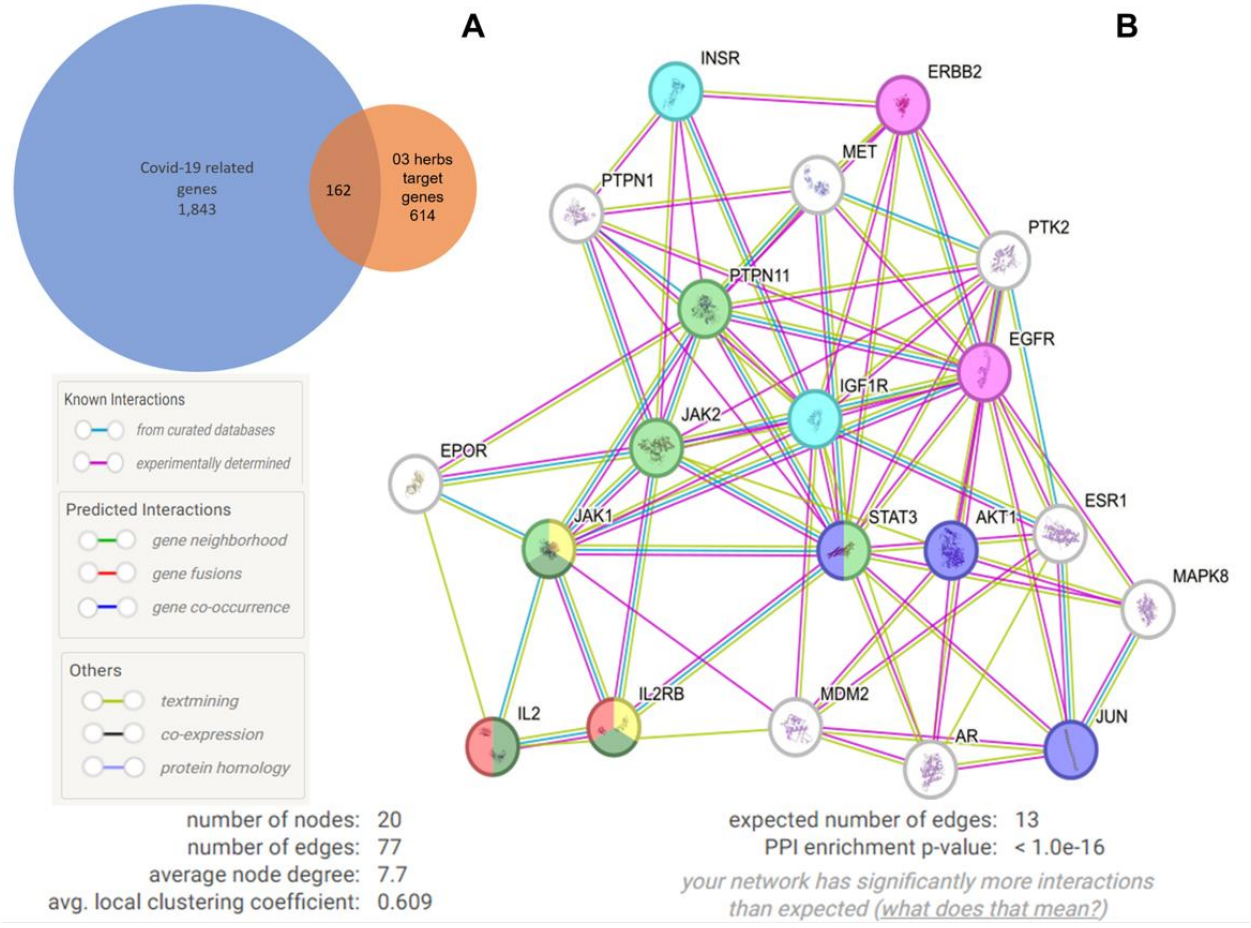

Figure 2: Analysis result of interfering target genes and protein-protein interaction network

3.3 Gene Ontology analysis

Analyzing 162 genes on the DAVID bioinformatics tool obtained Gene Ontology analysis. Functional GO analysis for biological process, results in **Figure 3** suggested that the mechanism of action of the compounds is strongly related to the signal transduction process, followed by the active coordination of the activities of the RNA transcription enzymes. Biological processes that are less affected include the response to drugs, downregulation of programmed cell death (apoptosis), and protein autophosphorylation. These processes hold significance in the context of COVID-19, as inflammation and cytokine storms are linked to multiple signal transduction pathways (Yang *et al*., 2021). Viral replication is strongly influenced by RNA transcription. Additionally, apoptotic cell death is also a previously reported phenomenon with CD4 and CD8 cells, related to the COVID-19 disease and strongly affecting disease prognosis (Choudhary *et al*., 2021, V'Kovski *et al*., 2021). The outcomes of the Gene Ontology (GO) analysis have offered insights into potential biological processes influenced by the investigated compounds and their relevance to COVID-19 pathology. Noteworthy information suggesting further experimental research on these compounds includes the activity of RNA transcription enzymes and the mechanism of programmed cell death of immune cells.

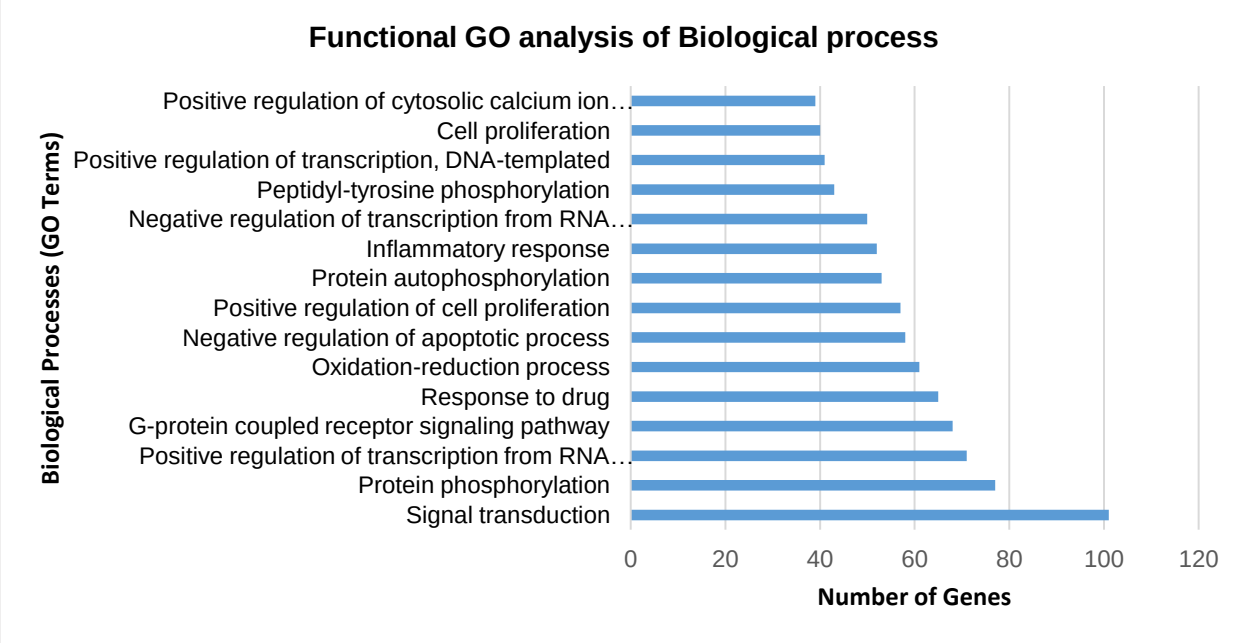

Figure 3: Gene Ontology analysis result of biological process

To further clarify the results of the biological process analysis, which demonstrated a relationship with the signal transduction process, GO of cellular component was analyzed. The results from **Figure 4** showed that the screened compounds may affect many different components of the cell, focusing on the plasma membrane, integral component of membrane and in the cytoplasm.

These results suggested a relationship between the biological effects of the compounds and the process of virus penetration through the host cell membrane, interaction with membrane receptor elements (integral component of membrane) and the entire membrane structure (plasma membrane), followed by the process of copying and multiplying viral RNA in the cytoplasm and nucleus (cytoplasm and nucleus). When SARS-CoV-2 approaches the cell membrane, the spike proteins (S proteins) of virus recognize and bind to the ACE2 receptor, and consequently transmit a signal to the serine protease TMPRSS2 on the cell surface to promote the viral invasion process, which in turn causes downregulation of ACE2 – this is also associated with inflammatory responses and cytokine storms. Therefore, the GO analysis results of cell components suggest that the 17 compounds screened above possibly produce biological activities through direct or indirect effects on the invasion process. Virus integration and replication require interaction with cellular components as abovementioned.

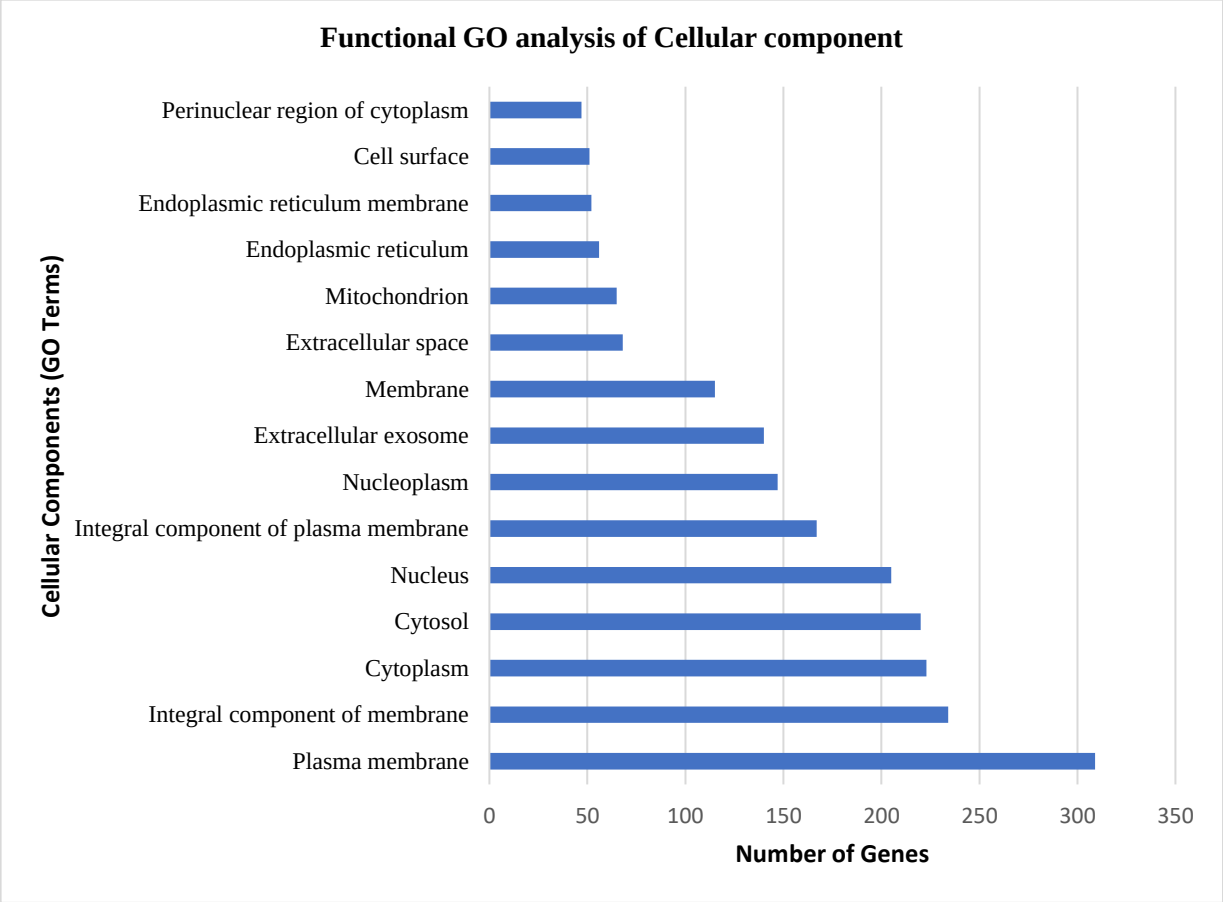

Figure 4. Gene Ontology analysis results of cell component

To further explore more possible activities of selected bioactive compounds at the molecular level, the functional GO analysis of the molecular function was conducted. **Figure 5** showed that the compounds with the highest biological interaction ability are concentrated in the protein binding

mechanism. Other biological interactions including ATP binding and metal ion binding also have a close relationship with the activity of proteins when it is necessary to mobilize energy from ATP and the mechanism that the activity of metalloproteins requires the presence of metals in the structure. These interactions help the SARS-CoV-2 virus enter cells by binding to proteins. Specifically, the protein binding interaction between the ACE2 receptor and the spike protein (S protein) of the SARS-CoV-2 virus is shown through the bridges between amino acids (Jiang *et al*., 2020, Gupta *et al*., 2020).

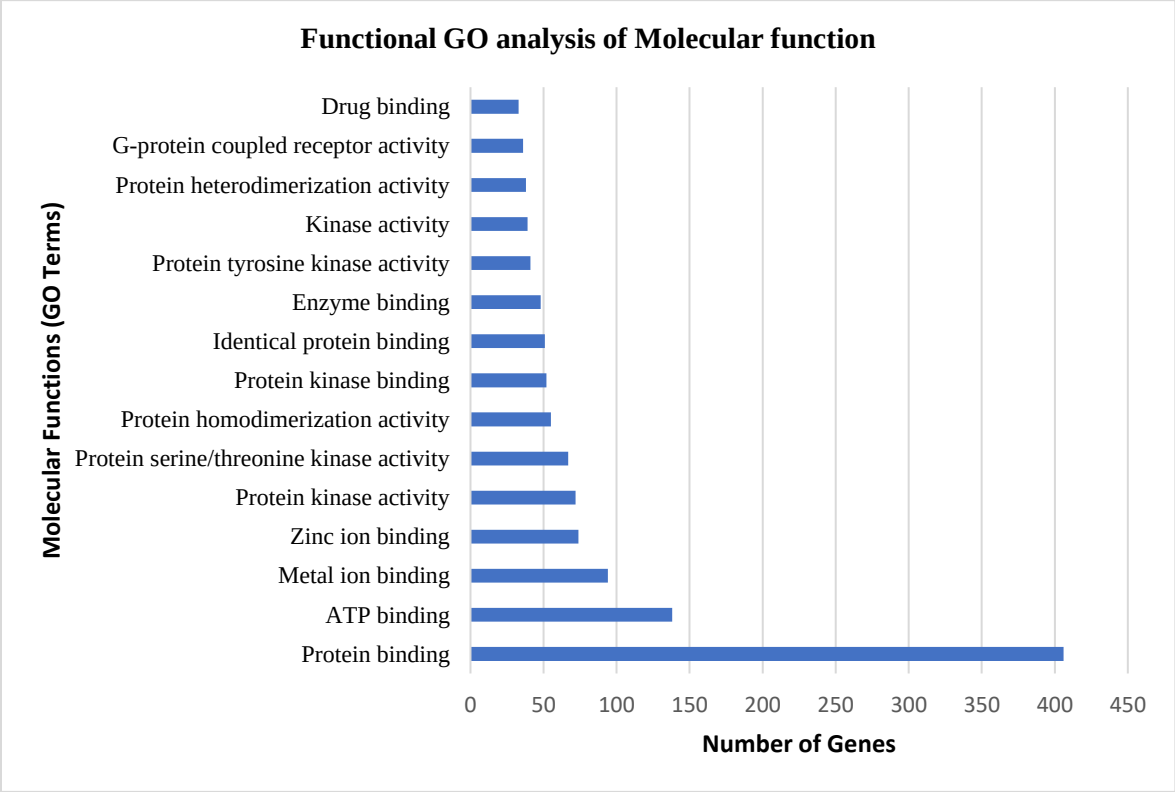

Figure 5. Gene Ontology analysis results of molecular function

3.3. KEGG signaling pathway analysis (pathway KEGG)

Table 2. Potential targets of 17 bioactive compounds at cellular level

Cell compartment	Target name
Extracellular	F2 (Thrombin); FXIII; FXIIIa; ACE; TNFα
Cell membrane	ATR1; ADAM17; TNFR; EGFR; TLR2/4; C3AR1
Intracellular	NFkB; MMP-3; IL-8; IL-2; IkB; Jak1; STAT3; SYK; PI3K; PLCγ; PKC; MAPK; TAK1; IKK; JNK; AP1; NLRP3; CASP1; TLR 7/8; TBK1; TNFα

The results of KEGG signaling pathway analysis showed that there are many diverse signaling pathways with 32 potential targets that have been highlighted (**Supplementary Figure S1**) that represent the mechanism of inflammation and cytokine storm in pneumonia caused by SARS-CoV-2. These targets are distributed not only extracellulary, but also at the cell membrane and in the

cytoplasm. Of these, there are 5 potential extracellular targets. These targets (**F2 (Thrombin); FXIII; FXIIIa; ACE; TNFα**) include factors related to blood clotting and signal information transmission, in which the ACE target is important in pathways related to the blood system, renin-angiotensin system through penetration and binding to the ACE2 receptor of the SARS-CoV-2 virus (Jiang *et al*., 2020, Gupta *et al*., 2020). There are 6 potential targets at the cell membrane (**ATR1; ADAM17; TNFR; EGFR; TLR2/4; C3AR1**) and the majority are also related to virus entry into cells and related to protein binding, which is consistent with the results of functional GO analysis of cellular components and molecular function (Yang *et al*., 2021, Palacios *et al*., 2021, Mikulicic *et al*., 2019). There are 21 potential targets that bearing the functions of activating inflammatory pathways and cytokine storms in the cytoplasm, demonstrating the diversity of cell death signaling pathways (**Table 2**). While bioinformatics analyses provide crucial insights into the potential therapeutic targets, the results must be validated through *in vitro* and *in vivo* studies to confirm the efficacy of these compounds against SARS-CoV-2.

4. Conclusion

In conclusion, the bioinformatics and network pharmacology analysis suggest that bioactive compounds from radix *Angelica dahurica*, *Chuanxiong* rhizoma, and *Cyperi* rhizoma may act synergistically against COVID-19 through interactions with key biological pathways and proteins associated with inflammation and viral entry. Future studies should focus on validating these findings experimentally to confirm the therapeutic potential of this herbal combination.

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Evaluation of standard operating procedure in Tabanan regional general hospital (RSUD Tabanan): usage of restricted antimicrobials

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Abstract

Background: The healthcare sector, specifically hospitals, is vulnerable to antimicrobial resistance due to diverse services and patient populations, alongside continuous intensive antimicrobial use. This resistance presents a significant national and global health threat. Therefore, implementing an antimicrobial stewardship program is crucial for reducing resistance development and spread in hospitals. Pharmacists, as healthcare professionals, have an essential role in upholding stewardship through pharmaceutical services in line with Standard Operating Procedure (SOP).

Objective: This study aimed to evaluate Restricted Antimicrobial Standard Operating Procedure (SOP-A) at Tabanan Regional General Hospital (RSUD Tabanan) to enhance the quality of pharmaceutical services.

Method: A qualitative method was used in SOP evaluation, comparing data from interviews with the Head of Pharmacy Installation at RSUD Tabanan and observations of implementation. The data were then contrasted with recent literature and regulations to identify discrepancies necessitating improvements. Moreover, data were analyzed using the descriptive method and presented in narrative, figure, and table formats.

Results: The results showed that based on interviews and observations of SOP-A implementation, several revisions were needed. These revisions include adding restricted antimicrobials from the reserve categories (cefepime, cefpirome, and carbapenems), establishing a protocol for eligible patients without waiting for culture results, outlining a process for obtaining approval from the Head of the Antimicrobial Resistance Control Program (PPRA) and Hospital Director, as well as incorporating antimicrobial usage history into the restricted antibiotic use application form.

Conclusion: SOP-A at RSUD Tabanan requires four key additions, namely a list of reserve category antimicrobials, a protocol for administering restricted antimicrobials to eligible patients without waiting for culture results, a process for obtaining approval from the Head of the PPRA and Hospital Director, as well as inclusion of antimicrobial usage history in the restricted antibiotic use application form.

Keywords: Antimicrobials, antimicrobial resistance, qualitative, restricted, standard operating procedure (SOP)

1. Introduction

The improper use of antimicrobial agents is a key contributor to the increasing incidence of antimicrobial resistance (CDDEP, 2021). For example, bacteria have various mechanisms facilitating resistance to antimicrobial activity, including genetic mutations that modify the active components of antimicrobials, thereby reducing efficacy. Additionally, certain bacteria can alter the outer membrane structures and receptors, preventing the binding of antimicrobials (Habboush & Guzman, 2023). These adaptive mechanisms allow survival of antimicrobial treatment and acquisition of resistance, which can be disseminated across species through horizontal gene transfer (Read & Woods, 2014). Antimicrobial-resistant infections represent a significant global health threat (ECDC, 2022), with this challenge being of particular concern in Indonesia.

The development of antimicrobial resistance is influenced by several factors, including widespread self-medication, unequal access to quality healthcare, a rising incidence of infectious diseases, and insufficient regulation of antimicrobial use (Parathon *et al.*, 2017). At the healthcare facility level, resistance is strongly associated with patterns of antimicrobial consumption, the accuracy of prescriptions, antimicrobial stewardship practices, and the perceptions of both consumers and healthcare providers concerning antimicrobial use (Limato *et al.*, 2022).

The high incidence of antimicrobial resistance frequently occurs in healthcare settings and is particularly common among patients with compromised immune systems, elderly individuals, and young patients who require frequent medical interventions (Pulingam *et al.*, 2022). Hospitals, as major providers of healthcare services, serve as potential hubs for the transmission of antimicrobial-resistant bacteria. This is attributable to the wide range of medical procedures performed, the diverse patient population, as well as the potential for prolonged and intensive use of antimicrobial agents (Knobler *et al.*, 2003).

World Health Organization (WHO) has implemented antimicrobial stewardship program that engages all healthcare workers in facilities, including hospitals, to mitigate the development of antimicrobial resistance (WHO, 2019). This program is designed to ensure that healthcare professionals follow appropriate antimicrobial usage protocols, thereby reducing the development and dissemination of resistance in healthcare environments (Bankar *et al.*, 2022). Pharmacists play a critical role in the implementation of antimicrobial stewardship in hospitals, with one of the key responsibilities being the development of local guidelines, such as Standard Operating Procedure (SOP) (Jantarathaneewat *et al.*, 2022).

SOP should be used by hospital pharmacists to enhance the delivery of pharmaceutical services. It serves as comprehensive guidelines that outline operational procedures in an organization, ensuring all decisions, actions, and the use of facilities are conducted effectively, consistently, and systematically by personnel (Tambunan, 2013). Technical, administrative, and procedural SOP are essential for guiding the implementation of healthcare services in hospitals (Taufiq, 2019). In particular, SOP related to antimicrobial use is critical for minimizing the incidence of resistance arising from antimicrobial treatment in healthcare settings, specifically hospitals. SOP must be continuously evaluated, taking into account the internal conditions of the hospital, the evolution of relevant policies in the region, and should be consistent with global health regulations to ensure the delivery of high-quality pharmaceutical services and enhance patient safety.

Tabanan Regional General Hospital (RSUD Tabanan) has established antimicrobial stewardship program in Pharmacy Installation, incorporating this initiative into SOP for restricted

antimicrobials issued in 2022. However, as new regulations regarding antimicrobial use develop over time, it is imperative to update SOP. Previous studies have assessed SOP related to the pharmaceutical supply planning process in Hospital X (Noviyani & Purnamasari, 2023), but there has not been an evaluation for restricted antimicrobials in hospitals. Therefore, this study aimed to evaluate Restricted Antimicrobial Standard Operating Procedure (SOP-A) in Pharmacy Installation of RSUD Tabanan to enhance the effectiveness and compliance with current standards.

2. Method

2.1. Data collection and processing methods

This qualitative study was conducted over 2 months, with permission (Number: 445/649/TIMKORDIK/RSUD/2023) and ethical clearance (Number: 445/634/TIMKORDIK/RSUD/2023) obtained from RSUD Tabanan. Data were collected through interviews with the Head of Pharmacy Installation at RSUD Tabanan and direct observations of SOP-A. The assessment was guided by current literature and regulations concerning restricted antimicrobials and pharmaceutical services in hospitals. The results from the interviews and observations were analyzed descriptively, using narratives, images, and tables to identify any compliance or non-compliance of activities or information in SOP-A relative to established guidelines. Any discrepancies identified indicate areas requiring improvement in SOP-A. This study concluded with the formulation of a revised SOP consistent with contemporary literature and regulations.

2.2. Tools and materials

Tools and materials used for data collection included SOP-A from RSUD Tabanan, a structured interview questionnaire, an observation sheet for documenting activities, an interview data collection form, informed consent forms, and relevant literature. These include *Peraturan Pemerintah Republik Indonesia No. 28 Tahun 2024 tentang Peraturan Pelaksanaan Undang-Undang No. 17 Tahun 2023 tentang Kesehatan* (Government Regulation No. 28 of 2024 regarding the Implementation Regulation of Law No. 17 of 2023 on Health), *Undang-Undang No. 17 Tahun 2023 tentang Kesehatan* (Law No. 17 of 2023 on Health), *Peraturan Menteri Kesehatan Republik Indonesia No. 28 Tahun 2021 tentang Pedoman Penggunaan Antimikroba* (Indonesia Minister of Health Regulation No. 28 of 2021 on Guidelines for Antimicrobial Use), and *Peraturan Menteri Kesehatan No. 8 Tahun 2015 tentang Program Pengendalian Resistensi Antimikroba* (Indonesia Minister of Health Regulation No. 8 of 2015 on the Antimicrobial Resistance Control Program (PPRA)). The results of

the interviews recorded using audio and video devices were subsequently analyzed descriptively with Microsoft Office software on a computer.

3. Result and discussion

In this study, an evaluation of SOP-A was carried out based on the results of interviews, observation of activities, and literature studies of applicable laws and regulations in Indonesia. Interviews with resource persons (the Head of Pharmacy Installation at RSUD Tabanan) and direct observation of antimicrobial service activities by RSUD Tabanan Pharmacy Installation have been carried out to obtain detailed information and a complete picture of SOP-A as well as the application. The results of interviews and observations of these activities are presented in **Table 1**.

Table 1. The results of interviews and observations of the implementation of sop-a

No.	Question	Resource person answer	Observation (appropriate/ inappropriate)
1.	Who are the parties engaged and responsible for preparing SOP-A?	The PPRA Team at RSUD Tabanan.	Appropriate
2.	Is there a set schedule for updating SOP-A? How long is the time?	Currently, there is no specific time for updating SOP-A. However, in every routine meeting at RSUD Tabanan, it is necessary to revise SOP-A and other applicable SOP, based on the current situation.	Appropriate
3.	Does SOP-A only cover the use of ceftazidime, meropenem, and vancomycin? Is there a possibility of adding other antimicrobials to the list of restricted antimicrobials in SOP-A?	At the time of SOP-A development, only ceftazidime, meropenem, and vancomycin were included based on the conditions at RSUD Tabanan and the current regulations. New regulations regarding restricted antimicrobials or other antimicrobials used in the hospital will be added to SOP-A.	Inappropriate
4.	Other than ceftazidime, meropenem, and vancomycin, what antimicrobials are currently used at RSUD Tabanan based on the newest list of restricted antimicrobials?	Cefepime, cefpirome, and the carbapenem class.	Appropriate
5.	Are there any additional documents required besides restricted antimicrobial form for prescribing and administering restricted antimicrobials at RSUD Tabanan? If yes, what documents are required?	Yes, in addition to restricted antimicrobial form, an additional document required is the culture result of the patient sample from the laboratory, which must be attached as part of the form. Culture sampling is a mandatory requirement for every patient who will receive restricted antimicrobials at RSUD Tabanan.	Appropriate
6.	What are the specific situations referred to in SOP-A that allow for the	Certain situations that require the administration of restricted antimicrobials without culture sampling include urgent	Inappropriate

No.	Question	Resource person answer	Observation (appropriate/ inappropriate)
	submission of restricted antimicrobials without culture sampling?	situations, such as in post-chemotherapy patients who show febrile neutropenia symptoms, but we have not included this situation in SOP-A.	
7.	What is the procedure for reporting the use of restricted antimicrobials by pharmacists to the PPRA?	Reporting has been performed through in-person meetings, phone calls, or electronic messages when the PPRA cannot meet. Culture results will be submitted later. This is carried out to ensure decisions on the administration of restricted antimicrobials can be made immediately according to the patient's condition.	Inappropriate
8.	Is there a special handling procedure when bacteria resistance is detected after administering restricted antimicrobials to the patient?	There have not been any cases of bacteria resistance after the administration of restricted antimicrobials, no special handling procedures have been designed for this situation. However, when this occurs in the future, there will be an evaluation and development of operational procedures according to current guidelines and recommendations.	Appropriate
9.	How is SOP-A implementation efficient in improving the quality of patient health services at RSUD Tabanan?	SOP-A plays an important role in controlling the use of antimicrobials at RSUD Tabanan, specifically in reducing the incidence of antimicrobial administration errors. For patients with certain conditions, restricted antimicrobial administration can be carried out without waiting for culture results, according to SOP-A guidelines. This allows for faster management of the patient health condition.	Appropriate
10.	Is the submission of restricted antimicrobial form to the PPRA required to provide information on the history of antimicrobial use?	Information on the history of antimicrobial use is essential for restricted antimicrobial application form, but it is currently not included.	Inappropriate

Description:
SOP-A: Restricted Antimicrobial Standard Operating Procedure in RSUD Tabanan

As shown in **Table 1**, four factors are not in accordance with the latest regulations, including no inclusion of other types of restricted antimicrobials, the absence of complete information about certain conditions where antimicrobials can be given before culture sampling, the absence of procedures or flows governing approval by the Head of the PPRA before pharmacists administer antimicrobials to patients, and the incomplete restricted antimicrobials form. Therefore, these four factors need to be added in making the new SOP for the Use of Restricted Antimicrobials. The results of these interviews and observations are then used as a reference to evaluate SOP-A.

According to *Peraturan Pemerintah No. 34 Tahun 2018 tentang Sistem Standardisasi dan Penilaian Kesesuaian Nasional* (Government Regulation No. 34 of 2018 concerning the National

Standardization and Conformity Assessment System), standards are optimized technical or appropriate requirements, including procedures and methods that are prepared based on the consensus or related government or international decisions by taking into account the requirements of safety, security, health, environment, development of science and technology, experience, as well as current and future developments to obtain the maximum benefit. Standards are made to direct service delivery and expected results. To produce performance in accordance with the established standards, a standardized set of procedures is needed, also known as SOP. In general, SOP in hospitals is a guideline or reference for carrying out tasks or work in line with the functions and performance assessment tools based on technical, administrative, and procedural indicators of the work procedures concerned (Wiraya and Haryati, 2022). In the implementation, SOP must always be reviewed to accommodate and anticipate changes related to the technical, administrative, and procedural indicators concerned.

One of SOP that has been implemented at RSUD Tabanan is SOP-A made by RSUD Tabanan on August 29, 2022. It was prepared based on Indonesia Minister of Health Regulation No. 8 of 2015 and *Surat Keterangan Direktur Rumah Sakit No.422/SK.RSUD/2022 tentang Penggunaan Antimikroba pada Rumah Sakit Umum Daerah Kabupaten Tabanan* (Hospital Director's Statement Letter No. 422/SK.RSUD/2022 concerning the Use of Antimicrobials at RSUD Tabanan) that refers to *Undang-Undang Republik Indonesia No. 36 Tahun 2009 tentang Kesehatan and Peraturan Menteri Kesehatan No. 72 tahun 2016 tentang Standar Pelayanan Kefarmasian di Rumah Sakit* (Indonesian Law No. 36 of 2009 concerning Health and Regulation of the Minister of Health No. 72 of 2016 concerning Standards of Pharmaceutical Services in Hospitals).

According to Indonesia Minister of Health Regulation No. 8 of 2015, antimicrobial resistance refers to resistance to antimicrobials that are usually effective in treating infections caused by bacteria, fungi, viruses, and parasites. Currently, the increase in cases of antimicrobial resistance continues to rise, while the rate of drug development has not been able to keep up with the development of this resistance. An optimal antimicrobial use management strategy is needed to prevent the development of resistance and improve patient outcomes. Restriction is a control effort by limiting the use of antimicrobials (Bardani, Andriani, and Rahmadevi, 2021). Grouping Restricted antimicrobials aims to prevent a more severe increase in resistance, considering the many bacteria that have developed resistance. On the other hand, the discovery of new, more effective antimicrobials has not been able to keep up with the rate of resistance development (Basavaraju *et al.*, 2021).

Following Indonesia Minister of Health Regulation No. 8 of 2015, antimicrobial groups that can be used in hospitals are divided into those free to be used by all clinicians (non-restricted), and others restricted to expert team approval (restricted and reserved). The regulation does not explain what types of antimicrobials are included in these groups. Therefore, to prepare SOP, the types of reserved antimicrobials have been determined based on the availability which is the last line to overcome bacteria infections in the hospital (Saleem *et al.*, 2020). Three types of antimicrobials that have been listed in SOP restricted antimicrobials category include ceftazidime, meropenem, and vancomycin. Based on Indonesia Minister of Health Regulation No. 28 of 2021 on Guidelines for Antimicrobial Use, there is a grouping of antimicrobials in line with the AWaRe (access, watch, and reserve) category also recommended by WHO (WHO, 2023), The existence of the latest regulations is then taken into consideration in improving SOP.

Access category antimicrobials are used for common bacteria infections and can be prescribed by doctors, dentists, specialists, and reviewed by pharmacists. Access category antimicrobials consist of amoxicillin, ampicillin, amoxicillin-clavulanic acid, ampicillin sulbactam, benzathine benzylpenicillin, doxycycline, erythromycin, phenoxymethyl penicillin, gentamicin, kanamycin, clindamycin (oral), cloxacillin, chloramphenicol, metronidazole, oxytetracycline injection, thiamphenicol, pyrimethamine, procaine penicillin, cefadroxyl, cephalexin, cefazolin, ciprofloxacin (oral), spiramycin, streptomycin, sulfadiazine, and tetracycline (Kemenkes RI, 2021).

Watch category antimicrobials are used for special indications or when access groups are ineffective and can be prescribed by specialist doctors, dentists, reviewed by pharmacists, and approved by an infection consultant doctor. When no infection consultant doctor is available, approval is given by a member of Antimicrobial Resistance Control Committee (KPRA). Antimicrobial watch category consists of amikacin, azithromycin, fosfomycin, clarithromycin, levofloxacin, moxifloxacin, netilmicin, ofloxacin, cefixime, cefoperazone-sulbactam, cefotaxime, cefpodoxime proxetil, cefazidime, ceftriaxone, cefuroxime, and ciprofloxacin injection (Kemenkes RI, 2021).

Reserve category antimicrobials reserved to treat bacteria infections caused by Multiple Drug-Resistant Organisms (MDRO) are the last resort in severe life-threatening infections and only prescribed by specialist doctors and dentists, reviewed by pharmacists, and approved for use by Antimicrobial Stewardship (PGA) team which is part of the Hospital KPRA. Antimicrobials in the reserved category consist of aztreonam, daptomycin, carbapenems, cotrimoxazole injection, linezolid, nitrofurantoin, piperacillin-tazobactam, polymyxin b, polymyxin e, cefepime, cefpirom, cefarolin, teicoplanin, tigicycline, vancomycin, cefolozane-tazobactam, and cefazidime-avibactam (Kemenkes RI, 2021).

SOP-A has been regulated regarding what types of antimicrobials are included in restricted antimicrobials, namely ceftazidime, meropenem, and vancomycin. However, following Indonesia Minister of Health Regulation No. 28 of 2021, there are still discrepancies. Three types of reserve category antimicrobials are still used at RSUD Tabanan which should be classified as restricted antimicrobials but have not been included in SOP, namely cefepime, cefpirome, and other carbapenem groups besides meropenem. This was discovered from the results of interviews delivered directly by the Head of Pharmacy Installation: "Cefepime, cefpirome, and carbapenems such as ertapenem, doripenem, and imipenem are also used as a treatment for infections at RSUD Tabanan but we have not included them in SOP". Therefore, the results of SOP evaluation indicate that the three types of antimicrobials need to be included in the new SOP-A, as shown in Appendix 1.

In reviewing the previous SOP-A, there are two types of flow for the administration of restricted antimicrobials, differentiated by the patient treatment room. In the first flow, when the prescription and drug request form originates from the attending physician for a patient in intensive care (ICU, HCU 1, HCU 2, Isolation ICU, NICU, Hemodialysis, or Declining Immune Room), pharmacy staff can prepare and dispense the prescribed restricted antimicrobials without requiring approval from the Head of the PPRA Team or Hospital Director. The evaluation shows that this first flow of SOP is appropriate and does not necessitate any changes. However, there are a few considerations for pharmacy staff. Before drug administration, the medical team should be consulted to obtain samples for complete blood tests when necessary. Interviews showed instances of non-compliance with culture testing before antimicrobial administration. Cultures are the gold standard for detecting and identifying bacteria infections, and the results are crucial for making informed decisions regarding the appropriate use of specific antimicrobials based on the infection source, thereby preventing the development of antimicrobial-resistant bacteria (Herman *et al.*, 2019).

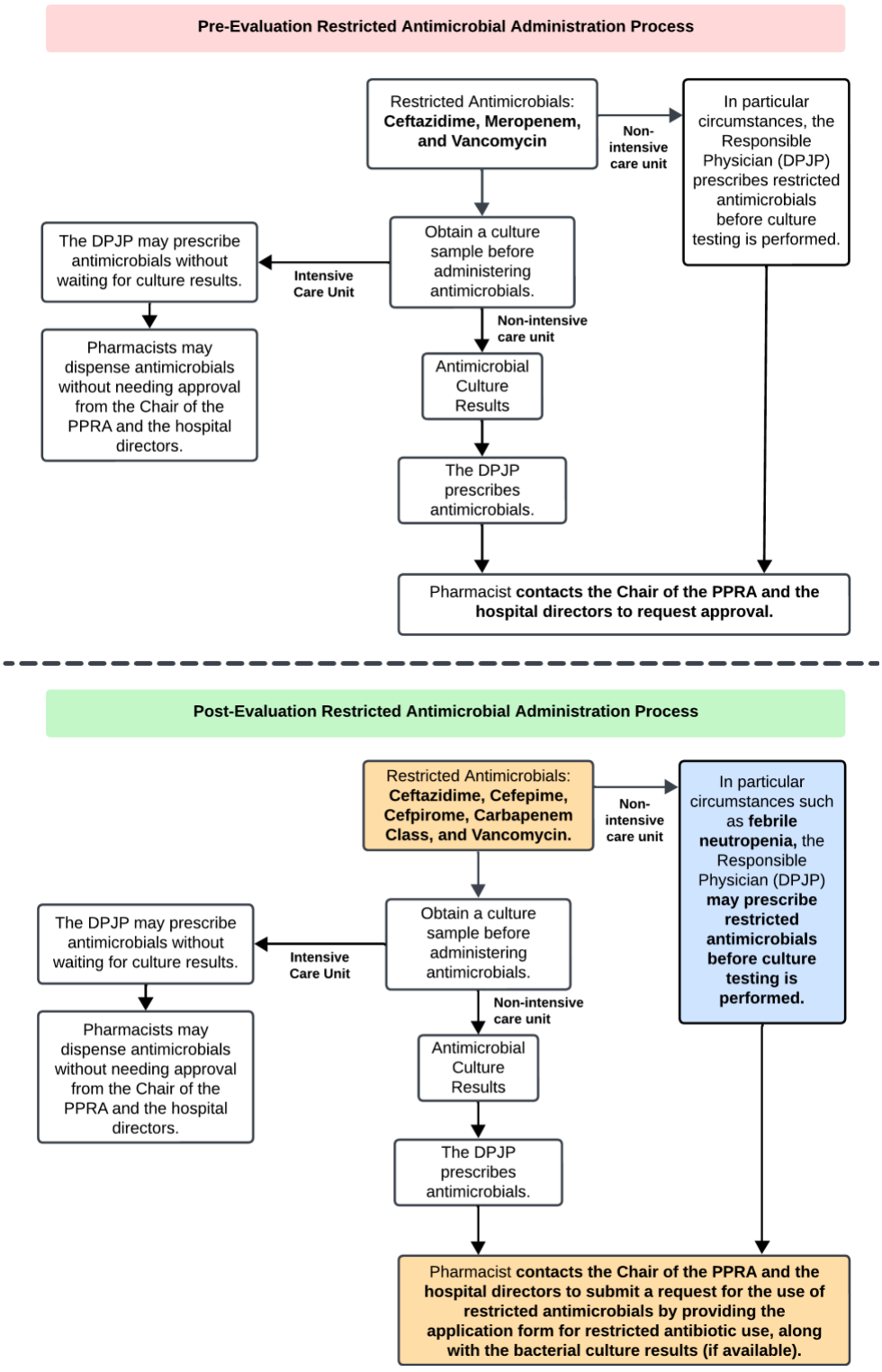
In the second flow, when the prescription and drug request form are issued by the attending physician for patients receiving treatment outside the intensive care unit, the administration of restrictive antimicrobials is only allowed after completing a blood test and culture. However, in certain urgent situations, pharmacists or pharmaceutical technicians may request special control antimicrobials by consulting with the Head of the PPRA and Hospital Director for approval before the culture examination. SOP does not clearly define the specific scenarios that may justify submitting requests for restricted antimicrobials before obtaining culture results. As stated by the Head of Pharmacy Installation in an interview: "Certain urgent situations necessitate the administration of restricted antimicrobials without waiting for culture results, specifically in post-chemotherapy patients showing symptoms of febrile neutropenia." In general, febrile neutropenia, a common side

effect of chemotherapy, is defined by a body temperature of ≥ 38°C and an absolute neutrophil count (ANC) of ≤ 500 cells/mm^3 due to the myelosuppressive effects of the treatment. This condition severely compromises the immune system, increasing susceptibility to bacteria infections, and when not addressed promptly, can delay further chemotherapy (Natawidjaja, *et al.*, 2019; Pratiwi *et al.*, 2022). Therefore, based on the evaluation of SOP-A, it is essential to incorporate specific situations that allow pharmacists or pharmaceutical technicians to administer special control antimicrobials without awaiting culture results, resulting in the creation of a new flowchart in the revised SOP-A (see Appendix 1).

After an interview with the Head of Pharmacy Installation, it was stated that "There is no special flow as pharmacists or pharmaceutical technicians contact the Head of the PPRA and Hospital Director to submit requests for restricted antimicrobials before culture examination, with the culture results to be proposed later." This indicates that there is currently no established protocol for requesting approval before submitting requests. The PPRA is managed by a specialized team responsible for formulating policies in hospitals. The primary tasks include establishing general policies and guidelines for antimicrobial use, conducting integrated case analyses regarding infectious disease management, and evaluating sensitivity to antimicrobials (Rukmini *et al.*, 2019). Therefore, SOP-A needs to establish a new protocol for pharmacists or pharmaceutical technicians to follow when contacting the Head of the PPRA and Hospital Director regarding requests for the administration of restricted antimicrobials. The revised process should include the submission of restricted antibiotic use application form, prepared by the attending physician, along with the culture results, when available, or a follow-up once the culture is in progress. Figure 1 shows differences in restricted antimicrobial administration flow before and after the evaluation, indicating the changes incorporated into the updated SOP-A (see Appendix 1).

The evaluation of SOP-A shows that the current restricted antibiotic use application form at RSUD Tabanan includes essential information such as the identities of the doctor and patient, patient diagnosis, treatment room, patient insurance, type and dosage of antimicrobial, duration of use, reason for administration, and microbial culture results. However, it lacks the patient antimicrobial use history, which is crucial for optimizing treatment outcomes. To address this gap, it is necessary to include antimicrobial usage history in restricted antibiotic use application form, as outlined in the new SOP in Appendix 1. This addition is critical because selecting appropriate antimicrobials requires considering bacteria sensitivity based on culture results, local resistance patterns, and the patient previous antimicrobial use (Adil & Kundarto, 2019).

In conclusion, all previously described evaluation results have been incorporated into the new SOP-A, as detailed in Appendix 1, enabling the implementation at RSUD Tabanan to enhance service quality and patient safety. The evaluation was conducted comprehensively, engaging informant interviews, direct observations, and comparisons with relevant literature as well as regulations to assess the suitability of SOP and implementation in practice. However, this study has limitations, as it relied on a single informant, the Head of Pharmacy Installation at RSUD Tabanan. To enhance data validity, direct observations of SOP-A implementation were also included. Future studies should adopt a similar comprehensive method to evaluate other SOP, engaging multiple informants for a broader perspective.



Description:

The process has not yet been implemented at RSUD Tabanan.

The process has been implemented at RSUD Tabanan, but it is not yet documented in SOP-A.

Figure 1. Comparison of restricted antimicrobial administration processes: pre- and post-evaluation

4. Conclusion

In conclusion, based on SOP-A evaluation, four key modifications were identified. First, the list of restricted antimicrobials must be updated to match the reserved category outlined in Indonesia Ministry of Health Regulation No. 28 of 2021. Second, a process should be introduced for administering restricted antimicrobials to patients with specific conditions without waiting for culture results. Third, an approval process requiring authorization from the Head of the PPRA and Hospital Director should be added. Finally, antimicrobial usage history should be included in restricted antibiotic use application form. These changes had been incorporated into the updated SOP-A.

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Appendix

Appendix 1. Recommendations for the Restricted Antimicrobial Use Standard Operating Procedure (SOP-A)

	Restricted Antimicrobial Use		
	Document No. xx.xx.xxxx/xxx	Revision No. xx	Page 1/2
STANDARD OPERATING PROCEDURE (SOP)	Date of Issue date - month - year	ESTABLISHED BY THE HOSPITAL DIRECTOR Director's Name Employee ID No.: xx.xxx.xx	
DEFINITION	Restricted antimicrobials are a group of antimicrobials whose use is limited and requires approval from the Antimicrobial Resistance Control Program Committee of RSUD Tabanan.		
OBJECTIVES	To serve as a reference for implementing steps to: 1. Ensure the quality of antimicrobial use in accordance with antimicrobial usage guidelines, hospital formulary, and clinical practice guidelines. 2. Achieve prudent use of antimicrobials. 3. Inhibit the emergence of resistant normal flora. 4. Minimize treatment costs and healthcare services.		
POLICIES	1. PERMENKES No. 8 Tahun 2015 Tentang Program Pengendalian Resistensi Antimikroba (Minister of Health Regulation No. 8 of 2015 concerning the Antimicrobial Resistance Control Program). 2. PERMENKES NO. 28 Tahun 2021 Tentang Pedoman Penggunaan Antimikroba (Minister of Health Regulation No. 28 of 2021 concerning Antimicrobial Use Guidelines). 3. SK Direktur NO.422/SK/RSUD/2022 Tentang Penggunaan Antimikroba Pada Rumah Sakit Umum Daerah Kabupaten Tabanan (Director's Decree No. 422/SK/RSUD/2022 concerning Antimicrobial Use in RSUD Tabanan).		
PROCEDURE	1. Restricted antimicrobials may only be prescribed by the Responsible Physician (DPJP), unless there is an instruction or delegation to the on-duty doctor/Manager on Duty. The restricted antimicrobials include: a. Ceftazidime c. Vancomycin e. Cefepime b. Carbapenem group d. Cefpirome 2. In intensive care areas (ICU, HCU 1, HCU 2, Isolation ICU, NICU, Hemodialysis, and Immune Compromised Room (ICR)), the administration of restricted antimicrobials is as follows: a. Collect a sample for Complete Blood Count (CBC) testing (if not previously conducted).		

Appendix 1. Recommendations for the Restricted Antimicrobial Use Standard Operating Procedure (SOP-A) (Continued)

<table>
<tr><td rowspan="2"> 
</td><td colspan="3">Restricted Antimicrobial Use</td></tr>
<tr><td>Document No.
xx.xx.xxxx/xxx</td><td>Revision No.
xx</td><td>Page
2/2</td></tr>
<tr><td>STANDARD OPERATING PROCEDURE (SOP)</td><td>Date of Issue
date - month - year</td><td colspan="2">ESTABLISHED BY THE HOSPITAL DIRECTOR

<u>Director's Name</u>
Employee ID No.: xx.xxx.xx</td></tr>
<tr><td>PROCEDURE</td><td colspan="3">b. Collect a sample for culture testing before the Responsible Physician (DPJP) prescribes antimicrobials without waiting for culture results.
c. Pharmacists may dispense antimicrobials according to the prescription service procedure without needing to obtain approval from the Chair of the Antimicrobial Resistance Control Program Committee (PPRA) and the Board of Directors.
3. Apart from intensive care areas (ICU, HCU 1, HCU 2, Isolation ICU, NICU, Hemodialysis, and Immune Compromised Isolation Room (ICR)), the administration of restricted antimicrobials is as follows:
a. Collect a sample for Complete Blood Count (CBC) testing (if not previously conducted).
b. Collect a blood sample for culture testing.
c. The Responsible Physician (DPJP) may prescribe restricted antimicrobials after culture results are available.
d. In particular situations, such as febrile neutropenia, the DPJP may prescribe restricted antimicrobials before culture testing, while the pharmacist contacts the PPRA Chair and the Hospital Director.
e. To obtain approval for restricted antimicrobials prior to dispensing, the pharmacist must submit the restricted antibiotic use application form and, if available, the culture results to the PPRA Chair and Hospital Director.
f. Prescriptions for restricted antimicrobials must be accompanied by the restricted antibiotic use application form.</td></tr>
<tr><td>RELATED INSTALLATIONS</td><td colspan="3">1. All Medical Staff Groups (KSM)
2. Intensive Care Unit
3. Inpatient Care
4. Emergency Department (ED)
5. Outpatient Care
6. Pharmacy Department
7. Laboratory</td></tr>
</table>

Appendix 1. Recommendations for the Restricted Antimicrobial Use Standard Operating Procedure (SOP-A) (Continued)

RESTRICTED ANTIBIOTIC USE APPLICATION FORM

The undersigned,

Name : ……..

Provides the following patient information

Patient Name : ……..

Medical Record No. : ……..

Gender : ……..

Age : ……..

Diagnosis : ……..

Ward (Patient Room) : ……..

Insurance : ……..

Certifies that the patient genuinely requires:

Medication (Drug Name) : ……..

Duration : ……..

Dosage : ……..

Reason for Restricted Antimicrobial Administration : ……..

History of Antimicrobial Use : ……..

Microbial Culture Results : ……..

Acknowledged by,

Chair of the PPRA Committee	Deputy Director of Services/Deputy Director of Support	Attending Physician
(Name)	(Name)	(Name)

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Prevalence of depression among diabetes mellitus patients at primary health centers in Sleman

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Abstract

Background: Depression is a common psychological disorder in diabetic patients that potentially causes non-adherence and complications. However, information regarding the prevalence and risk factor of depression in diabetic patients in Indonesia remains inconsistent.

Objective: This study is aimed to determine the prevalence of depression and its associated factors among patients with type II diabetes mellitus (T2DM) in primary public health centers.

Method: This cross-sectional study involved adult patients with T2DM in primary health centers in Sleman, Indonesia. Subjects were recruited using the purposive sampling method, and depression was assessed using the PHQ-9 questionnaire. The association between subject characteristics and depression was determined using the chi-square of Fisher's exact test.

Results: Among 268 subjects, most of them were women, aged <60 years old, had at least 1 comorbidity, and were taking a combination of oral antidiabetics. More than half of subjects had T2DM for <5 years. The prevalence of depression among T2DM was 36.6%. Being aged ≥60 years old, having a low education level, being a provider of family, and having T2DM for 5-10 years ($p<0.05$) were significantly associated with depression.

Conclusion: T2DM patients aged ≥60 years old, who have a low education level, are providers of family, and have had T2DM for 5-10 years are more vulnerable to depression, thus needing more attention from healthcare providers to achieve diabetes goal therapy.

Keywords: depression, PHQ-9, risk factor, primary health care, diabetes mellitus

1. Background

Diabetes Mellitus (DM) is one of the chronic diseases with a considerable impact on the sufferers. This degenerative disease affects not only the elderly and middle-aged population but also the young adult population. In 2021, as many as 536.6 million people in the world suffered from DM. This number is expected to increase and reach 783.2 million people by 2045 (Sun *et al.*, 2022). Having 19.5 million people diagnosed with DM, Indonesia ranked 5th among the countries with the highest number of DM patients in 2021. By 2045, the number of Indonesia's population suffering from DM is predicted to increase to 28.6 million people (Sun *et al*., 2022).

Diabetes Mellitus affects not only the physical condition but also the psychological condition of the sufferers. People with DM are vulnerable to experiencing psychological disorders due to various factors. Depressive disorders or symptoms are quite common among DM patients (Akhaury & Chaware, 2022). The incidence of depression in people with DM can reach 2-3 times more frequently compared to the population without DM. Diabetes Mellitus and depression are two diseases that affect each other or have a bidirectional correlation. Hyperglycemia that lasts for a long time will lead to structural changes in the brain and trigger the neurodegenerative process. On the other hand, depression can trigger the hypothalamic-pituitary-adrenal axis (HPA-axis) system, or

what is often called the stress pathway, and the sympathetic nervous system. As a result, the production of cortisol in the adrenal cortex and adrenaline as well as noradrenaline in the adrenal medulla will increase, leading to insulin resistance and type-2 DM (Bădescu *et al*., 2016; Dziurkowska & Wesolowski, 2021). In the presence of such mechanism, depression can interfere with the glycemic control in people who already suffer from DM (Moshomo *et al*., 2022). In addition, DM patients with depression tend to be noncompliant with DM treatment regimen and healthy lifestyle recommendations, thus posing a risk of therapy failure (Lunghi *et al.*, 2017; Sumlin *et al*., 2014; Yang *et al*., 2023). As an example of non-compliance, DM patients who show symptoms of depression often unilaterally discontinue medication without consulting or discussing first with the physician (Lunghi *et al*., 2017). If non-compliance occurs over a long period of time, it will lead to various complications, including macrovascular and microvascular complications (Nouwen *et al*., 2019).

According to this explanation, it is necessary to perform a screening for assessing the symptoms of depression in DM patients, thus allowing health workers to decide further action to prevent serious symptoms of depression and optimize DM therapy. However, with the limited resources available at public health centers, it is necessary to prepare a priority scale to determine which patients need screening first. Therefore, it is necessary to conduct a study to assess which characteristics of DM patients show the potential for depression.

Several studies have been conducted to look for patient factors related to the incidence of depression. However, previous studies have found different results, and some studies were single centered. Single-center studies are less likely to represent the characteristics of DM patients comprehensively. In addition, the assessment instruments used in prior studies to measure the symptoms of depression had a fairly large number of question items, such as the Beck Depression Inventory (BDI), making them less suitable for primary health care facilities that require rapid screening (Putri *et al*., 2023; Saleh *et al*., 2020; Shofiyati, 2020). This study complements previous studies by using the multi-center approach and the Patient Health Questionnaire-9 (PHQ-9) instrument which is more in line with the setting of primary health care facilities. According to this, this study aims to determine the prevalence of patients who experience depressive disorders and to analyze the factors related to depression in DM patients.

2. Method

2.1. Research design

This study was an analytical observational study with a cross-sectional design. The research was conducted in several primary health centers in Yogyakarta. The research procedures had been

approved by Medical and Health Research Ethics Committee of the Faculty of Medicine of Universitas Islam Indonesia with the letter No. 15/Ka.Kom.Et/70/KE/V/2021. During the recruitment process, each prospective participant received an explanation about the data to be collected, and there was no research procedure that endangered the participants. To ensure the confidentiality of patient data, the interviews were conducted in a separate room. The prospective participants who were willing to be the subjects of the research then signed an informed consent.

2.2. Research subjects and sampling methods

The population of this study was type-2 DM patients in primary health centers. Meanwhile, the subjects of this study were the patients who met the inclusion criteria, which included adult patients (≥18 years old) who had been diagnosed with DM for at least one year and were willing to become participants. Patients with severe psychiatric disorders that limited them from interpreting the questions in the questionnaire and patients with impaired verbal communication were excluded from the study. The sampling technique chosen in this study was the non-random sampling with a purposive sampling design.

2.3. Research instrument

The depressive disorders in DM patients were assessed by filling out the Indonesian version of the PHQ-9 which had been validated by Dian *et al.* (2022). This questionnaire consists of 9 questions (**Table 1**). The subjects were asked to answer each question item with never (score: 0), several days (score: 1), more than half of the time in question (score: 2), or almost every day (score: 3) according to their own conditions. The total score was obtained by adding the scores of each question item. In assessing depressive disorders, the researcher referred to the interpretation of the previous research conducted by Kroenke *et al*. (2001). The subjects were categorized into depressed patients (total score: ≥10) and non-depressed patients (total score: <10). The data reporting form was used to summarize the patient information related to the demographic characteristics and clinical characteristics data.

Table 1. Quetionnaire PHQ-9

Over the last 2 weeks, how often have you been bothered by any of the following problems?	Not at all	Several days	More than half the days	Nearly everyday
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed or hopeless	0	1	2	3
3. Trouble falling asleep pr sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3

Over the last 2 weeks, how often have you been bothered by any of the following problems?	Not at all	Several days	More than half the days	Nearly everyday
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed or the opposite – being so figety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself	0	1	2	3

2.4. Data collection procedures

The data collection process was carried out prospectively. The patient recruitment was conducted at the Pharmacy Installation of the primary health centers in Sleman after the patients received their medicine. The researcher explained the research procedures and the guarantee of confidentiality of the data obtained. The questionnaire was filled out after the patients agreed to be the subjects of the study and signed the informed consent. The direct interviews with the research subjects were conducted to obtain data on their demographic characteristics (age, gender, level of education, and source of income) and clinical characteristics (duration of DM, family history with DM, comorbidities, smoking habits, and oral antidiabetic therapy modalities). The clinical characteristics data on the weights and heights was obtained from the results of the last measurement at the polyclinic. The data was then recorded in the case report form. The weight and height measurements were carried out by nurses to calculate the body mass index (BMI). The body mass index is obtained by calculating the ratio of the body weight (kg) to the square of the height (m^2) and is categorized into underweight, normal weight, overweight, and obesity based on the WHO classification.

2.5. Data analysis

The data obtained was then analyzed using SPSS version 23. Frequencies were used to describe demographic characteristics, clinical characteristics, and depressive disorders. Meanwhile, the Chi-squared test or Fisher's exact test was used to assess the significance of the correlation between the independent variables (demographic characteristics and clinical characteristics and potential depressive disorders). The Fisher's exact test is used when the conditions of the Chi-squared test are not met; for example, there is a column with zero (0) value or more than 20% of the

expected value is less than 5. The correlation between two variables is significant if the value of *p* is <0.05.

3. Results and discussion

3.1. Characteristics of the research subjects

A total of 268 patients who met the inclusion and exclusion criteria were recruited as the study subjects and included in the statistical analysis. Based on **Table 2**, most of the research subjects were female, and the majority were <60 years old. More than half of the study subjects had low educational attainment or completed only the elementary school level. In addition, most of the study subjects relied on their spouses as the breadwinner.

Table 2. Socio-demographic characteristics of the research subjects

Demographics	Frequency (n)	Percentage (%)
Gender		
Male	76	28.4
Female	192	71.6
Age		
<60 years	174	64.9
≥60 years	94	35.1
Educational level		
Low (elementary school)	136	50.7
Moderate (high school)	98	36.6
High (Higher education)	34	12.7
Source of income		
Oneself	112	41.8
Spouse	135	50.4
Others	21	7.8

More than 50% of the study subjects had DM with a duration of less than 5 years and had no family history of DM. Most of the research subjects had comorbidities, either one or more than one comorbidity. More than 90% of the subjects did not have a smoking habit. All the study subjects used oral antidiabetics, and the majority used a combination of oral antidiabetics.

Table 3. Clinical characteristics of the research subjects

Variable	Frequency (n)	Percentage (%)
DM duration		
< 5 years	143	53.4
5–10 years	89	33.2
>10 years	36	13.4
Family history with DM		
Yes	116	43.3
No	152	56.7

Variable	Frequency (n)	Percentage (%)
Comorbidities		
None	77	28.7
1 Comorbidity	108	40.3
>1 Comorbidities	83	31.0
Smoking habits		
Yes	26	9.7
No	242	90.3
Body mass index (BMI)		
Underweight (<18.5 kg/m2)	15	5.6
Normal weight (18.5-24.99 kg/m2)	163	60.8
Overweight (25-29.99 kg/m2)	65	24.3
Obesity (≥30 kg/m2)	25	9.3
Oral antidiabetic modalities		
Monotherapy	118	44.0
Combination	150	56.0

3.2. Prevalence of depression in patients with diabetes mellitus

The prevalence of depression in type-2 DM patients in the primary health centers assessed with PHQ-9 was 36.6% (**Table 4**). Several studies predicting the prevalence of depression in DM patients have been conducted in many developing and developed countries, showing different results. The research conducted by Qusaibi *et al.* (2022) dan Abuhegazy *et al.* (2022) on the population of Saudi Arabia showed that the prevalence of type-2 DM patients with depression was 54% and 36.6%, respectively. Meanwhile, the research in other developing countries, such as Ethiopia, resulted in a figure of 21.3% (Engidaw *et al.*, 2020). A multinational study involving 21 developing countries conducted by Aschner *et al.* (2021) on type-2 DM patients who used oral antidiabetics indicated that the prevalence of patients with depression was only 29%. Some other studies in developed countries showed much smaller prevalence compared to this study (<20%) (Jung *et al*., 2021; Salinero-Fort *et al*., 2018).

The result variation of these studies can be influenced by several factors, such as the socio-demographic conditions of each country, research instruments, research design, and size of the research sample. Several prior studies used the PHQ-8 instrument with a cohort design. Socio-cultural conditions and public perception of certain diseases can also play a role in triggering depression. People in some developing countries still have a negative perception of DM (Nabila *et al*., 2022) and have a fatal belief in DM (Al-Sahouri *et al*., 2019) thus increasing the potential of suffering depression. The level of knowledge also contributes to the depression incidence in DM patients (Holt *et al.*, 2014). The level of knowledge related to DM in the population of developing countries is still

relatively low compared to that in developed countries (Fottrell *et al*., 2018). Meanwhile, the level of knowledge will determine an individual's attitude and perception towards a certain disease which therefore has the potential to cause depression.

Table 4. Prevalence of depressive disorders in the study subjects

Variable	Frequency (n)	Percentage (%)
Depression	98	36.6
No depression	170	63.4

3.3. Factors related to depression

As presented in **Table 5**, the proportion of patients with depression in the male and female groups were almost similar (p>0.05). Several previous studies have found that gender is one of the predictors of depression in DM patients. In these studies, female DM patients were more likely to experience depression than male (Aschner *et al*., 2021; Salinero-Fort *et al*., 2018). Meanwhile, other studies have shown that gender has no effect on depression (Abuhegazy *et al*., 2022; Qusaibi *et al.*, 2022). Women are twice as likely to have depressive disorder than men because women experience hormonal fluctuations during their lifetime. These hormonal fluctuations mainly begin when women begin to experience puberty phase (Kuehner, 2017). In addition, the burden of various roles or demands toward women in society also contributes to increasing the risk of depression in women (Moshomo *et al.*, 2022).

In terms of age, the patients with the age of ≥60 years (the elderly) were significantly more potential to suffer from depression (p<0.05) (Table 5). This result is contrary to the previous research by Abuhegazy *et al*. (2022). In their study, the age group of <60 years tended to have a higher potential for depression. In general, the elderly were more vulnerable to experience depression compared to other age groups because their cognitive function and physical disability declined. In addition, they tend to experience various comorbidities (Szymkowicz *et al*., 2023). This condition will be worsen if the elderly patients experience anxiety over their illness, have conflicts with the family, and have financial problems or a lack of activities in society (Naviganuntana *et al.*, 2022). On the other hand, the elderly who receive emotional support and have a good perception of the quality of life will be able to avoid depression (Didoné *et al*., 2020). The difference between the results of this study and those of previous studies can occur because the subjects in the previous studies had a good perception of old age, thus enabling them to avoid depression (Karlin *et al*., 2016).

The socio-demographic aspects associated with potential depression were educational attainment and source of income (p<0.05) (**Table 5**). The highest percentage of the patients who had the potential to experience depression was found in the group of patients with low educational

attainment. It can be seen in **Table 5** that there was an inverse correlation between the level of education and depression. The higher the educational attainment of an individual, the lower the possibility of the individual suffering from depression. These results are in line with the research conducted by Patria (2022) in Indonesia. Higher educational attainment will positively correlate with a patient's literacy related to the disease that he suffers from, allowing him to better respond to his conditions (Hsu *et al*., 2020). However, other studies suggest that educational attainment increases the potential for depression (Chen *et al.*, 2013). This can occur due to the difference in the criteria of the research subjects. The research by Chen *et al*. (2013) only recruited patients who had just been diagnosed with DM while this study used subjects who had been diagnosed with DM for at least one year.

The patients who relied on themselves as the source of family income, or patients who became the breadwinner of the family, were more likely to suffer from depression (Table 5), which is in line with previous research (King *et al.*, 2020; Nene *et al.*, 2023). Patients who become the breadwinner bear the burden to meet the needs of their families. In addition, conflicts in the workplace, expectations for the job that does not match reality, and workload increase the tendency to experience depression (Nene *et al.*, 2023).

Table 5. Correlation between socio-demographic characteristics and depressive disorders

Demographics	Depression		No depression		*P* value
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)	
Gender					
Male	28	36.8	48	63.2	1.000
Female	70	36.5	122	63.5	
Age					
<60 years	55	31.6	119	68.4	0.022*
≥60 years	43	45.7	51	54.3	
Educational attainment					
Low	60	44.1	76	55.9	0.026*
Moderate	30	30.6	68	69.4	
High	8	23.5	26	76.5	
Source of income					
Oneself	56	50.0	56	50.0	<0.001*
Spouse	39	28.9	96	71.1	
Others	3	14.3	18	85.7	

* Statistically significant

According to **Table 6**, the patients who had been experiencing DM for 5-10 years were significantly more likely to suffer from depression compared to those in the other groups (p<0.05). However, the percentage of the patients with <5 years and >10 years of DM was less than that of patients with 5-10 years of DM. An increase in the duration of DM does not necessarily increase the

potential for depression, or an increase in the duration of DM is not linear with the potential to suffer from depression. There are variations in the results of previous studies. Previous research conducted by Almeida *et al.* (2016) has also found that the correlation between the duration of suffering from DM and the potential for depression is not linear. However, in the research by Almeida *et al*. (2016), the potential for depression decreased when the duration of DM reached 10-19.9 years then always increased along with the longer duration. Differences in the results of these studies can occur due to differences in the research subjects, sampling systems, research designs, and instruments used to assess depression. Meanwhile, the research conducted by Asefa *et al*. (2020) has shown that patients with DM for 5 years or more were twice more at risk of suffering from depression compared to those with less than 5 years of DM. The research conducted by Asefa *et al*. (2020) used the same research subjects, sampling methods, research designs, and research instruments as this study, but the difference in the classification of the duration of DM may have caused a difference in the results of the two studies. Another study conducted by Mokoagow *et al*. (2022) has shown that patients who have suffered from DM for more than 10 years tend to be more at risk of developing depression. However, the research by Mokoagow *et al*. (2022) involved COVID-19 patients as the subjects. These patients had different psychological conditions from DM patients without COVID-19 infection. The presence of a pandemic will increase patients' concerns about their health conditions, thereby increasing the risk of depression, especially if the patients have suffered from DM for a long time.

In this study, comorbidities were not significantly associated with potential depression (**Table 6**). This result is contrary to the previous research conducted by Mokoagow *et al*. (2022) and Sekhri & Verma (2023). In general, patients with comorbidities tend to be more susceptible to depression, especially if the comorbidities involve cardiovascular and nervous system disorders (Aljohani *et al*., 2021).

Oral antidiabetic modalities were also insignificantly associated with depression (**Table 6**). This result is supported by a previous study conducted by Kessing *et al*. (2020) which indicates that there is no difference in depression incidence between monotherapy and combination of oral antidiabetic users. Each type of oral antidiabetic has a different correlation with the incidence of depression in DM patients. The study conducted by Kessing *et al*. (2020) shows that the use of oral antidiabetics, either as monotherapy or in combination, especially in combination with metformin, may lower the incidence of depression. Other antidiabetics such as DPP4 inhibitors, GLP1 analogues, and SGLT2 inhibitors have also been shown to be associated with a reduced risk of depression in DM patients as highlighted in a study conducted by Wium Andersen *et al.* (2022).

Tabel 6. Correlation between clinical characteristics and depressive disorders

Variable	Depression		No depression		*P* value
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)	
DM Duration					
< 5 years	47	32.9	96	67.1	0.027*
5–10 years	42	47.2	47	52.8	
>10 years	9	25.0	27	75.0	
Comorbidities					
None	34	44.2	43	55.8	0.254
1 Comorbidity	37	34.3	71	65.7	
>1 Comorbidities	27	32.5	56	67.5	
Oral antidiabetic modalities					
Single	39	33.1	79	66.9	0.309
Combination	59	39.3	91	60.7	

* Statistically significant

3.4. Research limitations

This research is an observational study which therefore cannot determine or explain the causality between variables. Nevertheless, this study provides an overview of which factors have the potential to cause depression in DM patients. This study only classifies comorbidities based on the number, not based on the types. In fact, each comorbidity can pose a different risk of depression. This is the same case with the antidiabetic therapy modalities used. Not all antidiabetics are at risk of causing depression.

4. Conclusion

The prevalence of the symptoms of depression in the DM patients undergoing treatment in the public health centers in this study was 36.6%. Several factors, including age, educational attainment, source of income, and duration of suffering from DM correlated with the incidence of depression in the DM patients in the primary health centers. Further research is needed regarding the correlation between the type of comorbidities or the type of oral antidiabetics and the incidence of depression in DM patients.

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Stability and beyond-use date of anesthetic agents used in surgical procedures: a review

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Abstract

Background: Anaesthesia drugs are often divided into other syringes to be soluted or mixed with other medications to share with other patients for the sake of efficiency.

Objectives: This study aims to know the stability of anesthetic agents and the compatibility with co-simultaneous drugs used.

Methods: This review was conducted by searching literature through the following databases: PubMed, Science Direct, and Google Scholar. The keywords used in the search for articles were "stability," "beyond use date," "anesthetic drug," and "intravenous."

Results: The data showed that mixing fentanyl with levo-bupivakain or epinefrin is relatively stable up to one month, but it decreases only for 72 hours in dextrose 5% or normal saline (NS). Pethidin can be mixed with acetaminophen and metoclopramide using dextrose, NS, or water and stored up to 24 hours. Midazolam diluted in dextrose 5% (D5) or mixed with other medications maintains for stability up to 14 days or more. Stability of ketamine is 24 hours longer whether it is mixed in solvent or acetaminophen. Mixing with propofol induces instability because of the emulsion form of propofol.

Conclusion: In general, the anesthetic drugs of fentanyl, pethidine, midazolam, and ketamine are stable and safe for preparation and administration in more than 24 hours. These four medications are compatible with NS and D5 and all tested medications during 24 hours.

Keywords: stability, beyond use date, anaesthesia drugs, intravenous

1. Introduction

The administration of anesthetic medications is an integral component of surgical procedures. Their utilization aims to ensure the patient is pain-free and as comfortable as possible throughout the procedure. Accuracy of the dose and concentration of the drug plays a pivotal role to achieve this goal. However, the amount of injection drug concentration can change under conditions of instability; decrease of stability may decrease effectivity including the hemodynamic instability (Bedocs *et al*., 2019).

It is essential to recognize that anesthetics do not utilize a one-size-fits-all approach. Therefore modification such as dilution or mixing cannot be avoided for achieving personalised goal. Anesthetis often need to open, divide and store for several hours before administration. Sharing one dosage form for some patients and giving several medications at once can result in efficient anesthetic care (Bedocs *et al*., 2019). In addition, multidrug administration strategy often also can not be avoided in practical setting because of venous access limitations. Co-administration of anesthetic and other drugs in one pack may change the stability.

The stability of these anesthetic drugs including the "beyond use date" (BUD) is the date set on an opened sterile product when the state of the product is still within the stable range and can be delivered to the patient. When a sterile product is unsealed, it is exposed to the surrounding

environment and will change the stability (Herawati, 2012). The BUD is an indispensable metric in medical practice which is denotes the specific date set on an opened sterile product, signifying the period during which the product remains stable and can be administered safely to patients. This date is not chosen arbitrarily; it is based on extensive research establishing the period during which the product retains its efficacy and safety profile.

When the seal on a sterile product is opened, the product becomes susceptible to contamination from the surrounding environment. Humidity, light exposure, and ambient temperature can significantly impact a drug's stability. The BUD functions as a safeguard against the possible degradation of a drug's efficacy and safety, ensuring that patients receive medications that are both effective and free of contamination. Thus, the BUD serves multiple purposes. It does not only provide healthcare professionals with a clear guideline for the safe use of opened sterile products, but it also supports the overarching objective of medical practice, ensuring patient safety and providing optimal care. Adherence to the BUD and comprehension of its implications are essential for preserving the efficacy of medical treatments and ensuring patients' outcome.

As long as the medication is stored until the patient uses it, it is said to be stable if its properties have not altered physically, chemically, therapeutically, microbiologically, or toxicologically from those specified by the manufacturer (Noviani & Arrang, 2021). By selecting the right ingredients, compatible solvents, a secure storage location, and the ideal administration period, the risk of instability can be reduced (Husna *et al*., 2021). As a result, understanding the stability and BUD is critical for developing a safe anesthetic drug administration strategy. No publication has yet reviewed data on the stability and BUD of anaesthesia drug injection, to the best of the researchers' knowledge.

Understanding medications' stability and BUD is crucial, particularly when developing drug administration strategies. It is especially true for anesthetic medications, where even the smallest variation in stability can have significant clinical consequences. Notably, the stability and BUD are crucial for ensuring the safe and effective administration of anesthesia, given the importance of the surgeries and procedures that these medications facilitate. Surprisingly, despite the significance of this topic, no exhaustive literature review or publication has addressed the stability and BUD of anesthesia drug injections systematically. This research emphasizes the need for rigorous studies in this field to bolster the existing body of knowledge and establish best practices for anesthetic drug administration.

2. Method

2.1. Search strategy

Literature search had been done by using electronic sources or databases such as PubMed, Science Direct, and Google Scholar to find relevant literature, regardless of year. By selecting articles with an English language, a literature search was conducted. The keywords used in the search for

articles were "stability," "beyond use date," "anesthetic drug," and "intravenous." The literature search technique uses a combination of keywords with the boolean operators "OR" and/or "AND".

In order to facilitate an exhaustive review of the topic, a thorough literature search was conducted across multiple electronic databases, such as PubMed, Science Direct, and Google Scholar. The search strategy was not limited by publication date, ensuring that both classic and contemporary studies were proportionally considered. For ensuring consistency and clarity in the analysis, priority was given to articles predominantly composed in English. A keyword-driven search was conducted using terms such as "stability," "expiration date," "anesthetic drug," and "intravenous." The strategic use of boolean operators further improved the precision of the search: combining keywords using "OR" enabled the acquisition of a broader range of relevant literature, while the use of "AND" ensured the intersection of the primary themes under investigation. This methodological approach ensured a comprehensive and pertinent selection of articles to the subject of anesthetic drug stability and beyond-use dates.

2.2. Selection criteria

The inclusion criteria were articles that had been published in English and were available in full text; *in vitro* studies that discussed the stability of injections of anaesthesia drugs in combination with other drugs and solvents in certain storage conditions. The exclusion criteria were articles that did not include data on anesthetic drugs, solvents, other drug combinations, storage, and BUD.

In order to ensure the study's relevance and the collection of data, a precise set of inclusion and exclusion criteria was outlined. In terms of inclusion, the emphasis was placed on English-language articles, allowing for greater accessibility and comprehension among the research community. In addition, only complete studies were considered, allowing for a comprehensive analysis without the danger of omitted information. The emphasis was placed on *in vitro* studies as we delved deeper into the content details. These studies should focus on the stability nuances of anaesthesia drug injections, particularly when merged or combined with other substances or solvents. As this parameter plays a pivotal role in determining the drug's stability, particular storage conditions were also emphasized. In contrast, the exclusion criteria were developed to exclude articles that could dilute the review's focus. Articles were disregarded if they failed to provide data concerning anaesthetic drugs, their corresponding solvents, other potential drug combinations, and the conditions in which they were stored. In addition, articles that did not discuss the BUD of these anaesthetic drug mixtures were deemed extraneous and excluded. These stringent criteria aimed to collect a comprehensive, focused, and relevant dataset for the review. From the process of selection results the data as seen on **Figure 1**.

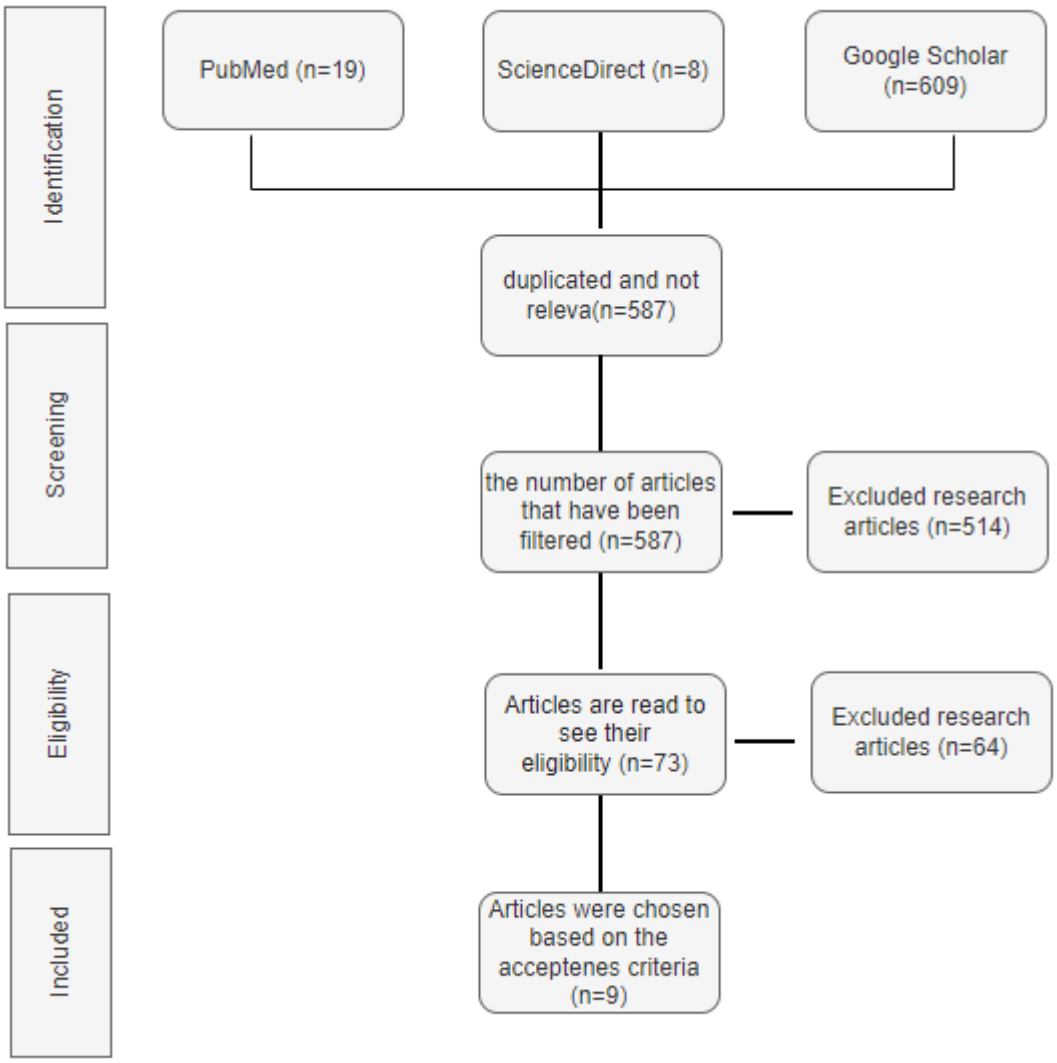

Figure 1. Diagram of retrieved studies

3. Results and discussion

The stability of the medication is a crucial factor in ensuring the safety and efficacy of the administered treatment. A drug is considered stable if its physical, chemical, therapeutic, microbiological, and toxicological properties do not deviate from the manufacturer's specifications during storage and up until the point of administration to the patient. The foundation for attaining this stability is frequently comprised of multiple factors. These include the careful selection of ingredients, the selection of compatible solvents, the designation of a storage environment that safeguards the drug from harmful external factors, and the determination of the optimal administration window. These measures guarantee the drug's efficacy and mitigate any potential risks associated with its instability

Selected research on the stability of anesthetic medications included 9 articles. Of these nine articles reported more than one medicine stability. Table 1 shows the stability of fentanyl identified from 4 articles. Meanwhile, the stability of pethidin was analysed from 3 articles as seen on **Table 2**. Then, midazolam stability was found in 4 articles and ketamine in 4 articles as shown on **Table 3** and **4** respectively.

Table 1. Stability of fentanyl with other drugs and solvent

No	Author, year	Drug	Solvent	Temperature	Duration assay	Stability
1	(Wilson *et al*., 1998)	Fentanyl (50µg/ml)	Sodium chloride 0,9%	5-38°C	7 days	7 days
2	(Wilson *et al*., 1998)	Fentanyl Midazolam	-	22-38°C	7 days	4 days
3	(Chen *et al*., 2020)	Fentanyl citrate (20 µg/ml) Naloxone hydrocloride (4 µg/ml)	Sodium chloride 0,9%	4°C or 25°C	72 hours	72 hours
4	(Helin-Tanninen *et al*., 2013)	Levo-bupivakaine Fentanyl Epinefrin	-	6°C	60 days	60 days
5	*(Helin-Tanninen et al*., 2013)	Levo-bupivakaine Fentanyl Epinefrin	-	22°C	60 days	40 days
6	(Helin-Tanninen *et al*., 2013)	Levo-bupivakaine Fentanyl	-	6°C	60 days	60 days
7	(Helin-Tanninen *et al*., 2013)	Levo-bupivakaine Fentanyl	-	22°C	60 days	40 days
8	(Hanifah *et al.*, 2020)	Fentanyl	Dextrose 5%	26-28°C	7 days	72 hours

Table 2. Stability of pethidine with other drugs and solvent

No	Author, year	Drug	Solvent	Temperature	Duration assay	Stability
1	(Hor *et al*., 1997)	Pethidine Metoclopramide	WFI	32°C	48 hours	48 hours
2	(Hor *et al*., 1997)	Pethidine Metoclopramide	Sodium chloride 0,9%	32°C	48 hours	48 hours
3	(Hor *et al*., 1997)	Pethidine Metoclopramide	Dextrose	32°C	48 hours	48 hours
4	(Hanifah *et al*., 2020)	Pethidine Acetaminophen	-	24-28°C	24 hours	24 hours

Table 3. Stability of midazolam with other drugs and solvent

No	Author, year	Drug	Solvent	Temperature	Duration assay	Stability
1	(Hanifah *et al*., 2020)	Midazolam Acetaminophen	-	24-28°C	24 hours	24 hours
2	(Xia & Chen, 2020)	Midazolam hydrocloride 0,5 mg/100mL Ramosetron hydrocloride 0,3mg/100mL	Sodium chloride 0,9%	4°C	14 days	14 days
3	(Xia & Chen, 2020)	Midazolam hydrocloride 0,5 mg/100mL Ramosetron hydrocloride 0,3mg/100mL	Sodium chloride 0,9%	25°C	14 days	14 days
4	(Hanifah *et al*., 2020)	Midazolam	Dextrose 5%	26-28°C	30 days	30 days

Table 4. Stability of ketamine with other drugs and solvent

No	Author, year	Drug	Solvent	Temperature	Duration assay	Stability
1	(Bedocs *et al*., 2019)	Propofol Ketamine	-	37°C	24 hours	24 hours
2	(Beiler *et al*., 2020)	Ketamine hydrochloride 2,5mg/mL	Sodium chloride 0,9%	28°C	48 hours	48 hours
3	(Hanifah *et al*., 2020)	Ketamine Acetaminophen	-	24-28°C	24 hours	24 hours
4	(Hanifah *et al*., 2020)	Ketamine	Dextrose 5%	26-28°C	7 days	7 days

General anesthesia is a medical state in which a patient is induced to become unconscious, lose sensation, and lose memory during surgical procedures or other medical interventions. It is a controlled and reversible condition that ensures the patient does not feel pain, discomfort, or become aware while undergoing a potentially painful or disturbing surgery (Brown *et al*., 2018).

Anaesthesia can successfully alleviate surgical pain. In a scenario with limited resources, using a single medicine to treat postoperative pain can help save money. Among the several advantages of anaesthetic include less post-operative bleeding, rapid gut function recovery, intact airways, and early detection of problems in awake patients (Arjumand et al., 2022).

Several research have used various combinations of solvents and storage temperatures to provide answers regarding the stability of anesthetic medicines. According to this study, anesthetic medications that have been opened frequently experience instability.

3.1. Stability of fentanyl with other drug and solvent

Fentanyl, like most therapeutically utilized opioids, exerts its pharmacological effects by activating the mu opioid receptor (MOR), which has a low affinity for the delta and kappa opioid receptors. Unlike morphine, which is an alkaloid produced from the opium plant, fentanyl is a synthetic, lipophilic phenylpiperidine opioid agonist. Fentanyl is a highly effective MOR agonist, with a binding affinity (Ki) of 1.35 nM for recombinant human MORs (Comer & Cahill, 2019a).

The use of fentanyl in surgery is often combined with various drugs. The combination of fentanyl with midazolam is often done by anaesthetists to achieve sedation and analgesia during surgery. Midazolam was discovered to work in tandem with fentanyl to induce anaesthesia (Ben-Shlomo *et al*., 1990). Fentanyl is frequently coupled with naloxone hydrochloride, with the goal of reducing fentanyl overdose use by reversing the effects of fentanyl-induced respiratory depression (Comer & Cahill, 2019b). When fentanyl is mixed with levobupivacaine, it causes an early start and prolonged duration of sensory and motor block, as well as surgical analgesia with stable haemodynamics and minimum side effects (Attri *et al*., 2015). Adrenaline, often known as epinephrine, has the potential to be utilized as an adjuvant in digital nerve blocks to expedite and prolong analgesic effects (Edinoff *et al*., 2021). Thus, mixing fentanyl, levo-bupivacaine and epinephrine is an alternative drug combination during surgery.

The stability of fentanyl is impacted by interactions with other medications, solutions, and temperatures. The longest stability at 6°C is 60 days for fentanyl with levo-bupivacaine, fentanyl, and epinephrine. The shortest stability is 3 days when fentanyl and naloxone hydrochloride are combined.

3.2. Stability of pethidine with other drug and solvent

Pethidine is the only opioid with adequate local anaesthetic activity to be used as the sole drug for spinal anaesthesia.The combination of opioid and local anaesthetic activity offers the ability to deliver surgical anaesthesia comparable to that obtained with traditional local anaesthetic drugs, as well as early postoperative analgesia that may be superior (Kee, 1998). The combination of pethidine with metoclopramide is often used during surgery. Metoclopramide is a well-known antiemetic that is widely used in PONV (Ilyas *et al*., 2017).

Similarly, the combination of pethidine and acetaminophen is frequently used in anaesthetic prescription drugs. Acetaminophen (N-acetyl-p-aminophenol, AAP), generally known as paracetamol, is a widely used antipyretic and analgesic that is widely regarded as an effective medication for pain and fever relief in adults and children (Park *et al*., 2014; Bateman *et al*., 2014; Jackson & Kapp, 2011).

The results of the pethidine-metoclopramide combination research dissolved with multiple different solvents, such as aqueus, sodium chloride 0.9%, and dextrose at 32°C temperature storage, with a test period of 48 hours, the results were stable 24 hours even with varied solvents. Pethidine-

acetaminophen combination at 24 – 28°C at 24 hours test duration without solvent, remained stable 24 hours.

3.3. Stability of midazolam with other drug and solvent

Midazolam is administered orally, intravenously, intranasally, and intramuscularly for sedation prior to diagnostic and therapeutic medical operations (Conway *et al*., 2021). Midazolam is a medicine used as an addition to regional and local anesthetic for a variety of diagnostic and therapeutic procedures, and it is well tolerated by both patients and physicians (Reves JG *et al*., 1985). A combination of midazolam and acetaminophen is often used in surgery, with the aim of achieving a sedative effect and controlling postoperative pain. While the combination of midazolam with ramosetron serves to obtain a sedative effect and reduce the onset of vomiting. Based on the results of the combination of midazolam with acetaminophen with a stable temperature of 24 - 28°C at a test duration of 24 hours, the combination of midazolam hydrochloride with ramosetron hydrochloride dissolved with sodium chloride 0.9% at a test duration of 14 days with storage temperatures of 4°C and 25°C remained stable for 14 days.

Midazolam's stability in the same solvent with various storage temperatures did not influence it, however midazolam's stability with various medication combinations did. Each drug has different storage temperature specifications; if the placement is not appropriate, it will affect the stability of the pharmaceutical preparation (Noviani & Arrang, 2021).

3.4. Stability of ketamin with other drug and solvent

Ketamine is a phencylidine class intravenous anaesthetic drug that stimulates the central nervous system. The use of ketamine at low doses is effective for standard opiod regimens and local anaesthesia (Riddell *et al*., 2019). Ketamine is a dissociative anesthetic drug that suppresses neuronal function in the cortex and thalamus and causes abnormal excitatory activity in the limbic system, including the hippocampus, resulting in electroencephalographic changes unique from those generated by anesthetics (Brown *et al*., 2010).

The use of ketamine is often combined with propofol, and acetaminophen. In surgery, the combination of ketamine and propofol seeks to improve anaesthesia. The combination of ketamine and propofol for procedural sedation and analgesia may be beneficial in theory, with the rationale being that using lower doses of each agent may result in a reduction of both agents' undesirable side effects while maintaining optimal conditions for performing procedures (Slavik & Zed, 2007). The combination of propofol and ketamine has the potential to produce superior sedation while being less harmful than either drug alone (Mortero *et al*., 2001). While the combination of ketamine and acetaminophen is to strengthen the pain of surgery. Acetaminophen is one of the most commonly utilized painkillers in operating rooms (Rahimzadeh *et al*., 2013).

The combination of these drugs, at a test duration of 24 hours, was observed to maintain its stability for 24 hours, even with different temperature storage. Different results when ketamine is only dissolved with 0.9% sodium chloride, with a test duration of 48 hours, the stability is maintained for the duration of the test.

3.5. Strength and limitation of the study

The review utilized a broad search strategy, including but not limited to PubMed, Science Direct, and Google Scholar, among other prestigious databases. This rigorous methodology was crucial in ensuring the extraction of relevant and comprehensive literature, paving the way for a comprehensive understanding of the topic. This exhaustive curation was further augmented by a detailed analysis illuminating the myriad factors contributing to drug stability. Elements such as storage temperatures, the nature of solvent mixtures, and various drug combinations were dissected to provide readers with a multifaceted understanding of the stability dynamics of anesthetic drugs. This investigation, with its theoretical and practical aspects, is of considerable importance. Understanding the subtleties of drug stability becomes crucial in surgical procedures, where anesthetic agents are indispensable. This research does not merely add to the body of knowledge; it also fills a glaring gap in the current literature by focusing on the stability and complex interactions of anesthetic agents, an area with significant clinical implications.

However, while the methodology and purpose of the study merit recognition, they have limitations. Primarily, the restriction to particular databases may introduce an unintended bias. The exclusive reliance on PubMed, Science Direct, and Google Scholar may overlook relevant studies that may exist in other academic repositories. In addition, the decision to limit the scope of the study to 17 anesthetic drugs raises an additional concern. Many medications are utilized in surgical contexts, and narrowing the focus could unintentionally limit the scope of the study's findings. In addition, there is the inherent difficulty of generalizing the study's results. Due to the study's limitations, caution must be exercised when applying its findings to various surgical environments, particularly in geographical regions with varying environmental variables. The lack of experimental validation is a significant and potential area for future research. Incorporating empirical validations would have added a tangible and more reliable dimension to evaluating drug stability and interactions despite the undeniable value of the insights gleaned from existing literature.

Conclusion

The anesthetic drugs of fentanyl, pethidine, midazolam, and ketamine are stable for 7 days, 2 days, 40 days, and 7 days respectively. This stability of anesthetic medicines may be impacted by medication also be impacted by the solvent used to dilute it. The results of compatibility study of fentanyl are safe to mix with NS, D5, Levo-bupivakain, and midazolam. Pethidine is compatible with

WFI, NS, D5, metoclopramid and midazolam. Midazolam is compatible with NS, D5, ramosetron and acetaminophen, and compatible is compatible with acetaminophen and propofol.

Conflict of interest

All researchers have stated that there are no potential conflicts of interest with this research, its authorship, and/or its publication.

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Quality of life and medical costs of dengue patients at PKU Muhammadiyah Hospitals in Yogyakarta and Bantul

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Abstract

Background: The manifestation of dengue is a decrease in hematocrit and platelet levels, leading to a decrease in the patient's quality of life and having an impact on the patient's medical costs.

Objective: This study objective was to ascertain the quality of life and medical costs associated with dengue patients.

Method: This study employed a cross-sectional design at PKU Muhammadiyah Hospitals in Bantul and Yogyakarta from January to August in 2023. Pediatric patients' quality of life was assessed using the EQ-5D-Y questionnaire, while adult patients' using the EQ-5D-5L questionnaire. Data on patients' medical costs was obtained from the hospital's financial database. The data were then analyzed descriptively and presented as a percentage of quality of life and average medical costs.

Results: The results showed that pediatric DF patients in both hospitals reported anxiety and depression, with percentages 100% (Yogyakarta) and 75% (Bantul), respectively. Meanwhile, most adult DF patients experienced problems in carrying out routines, of 71.4% (Yogyakarta) and 50% (Bantul), respectively. Adult DHF patients in both hospitals reported pain and discomfort of 66.7% (Yogyakarta) and 100% (Bantul), respectively. The highest average medical costs paid by DF and DHF patients at PKU Jogja Hospital were IDR 4,919,450 and IDR 6,981,500. DF and DHF patients at PKU Hospital with *BPJS* insurance, with the highest average medical costs of IDR 2,726,245.5 (for DF patients) and IDR 4,797,700 (for DHF patients) to cover for laboratory costs.

Conclusion: Dengue fever infections are impacting the patient's quality of life and medical costs.

Keywords: Dengue, medical costs, quality of life

1. Introduction

According to the *World Health Organization,* dengue is a disease caused by dengue virus infection (DENV), which is spread through mosquito bites that attack the human body (WHO, 2023). Dengue is classified into Expanded Dengue Syndrome (DSS), Dengue Hemorrhagic Fever (DHF), and Dengue Fever (DF) (WHO, 2011). This disease is transmitted to individuals of all age groups, including children, adults, to the elderly (Kemenkes, 2020). There are four serotypes of dengue viruses, namely DEN-1, DEN-2, DEN-3, and DEN-4, including the Arthropod-Borne Virus group, the genus Flavivirus, and the Flaviviridae family. In general, if a patient has been infected by one of the four types of dengue virus, the body will have immunity to the virus, but it does not guarantee immunity to the other three types of viruses (Lee *et al*., 2015; WHO, 2023). In 2023, the Indonesian Ministry of Health documented 28,576 cases of dengue diagnosed as DHF (Kemenkes, 2023). Meanwhile, Yogyakarta's health profile data in 2021 recorded 1187 dengue cases, with Bantul district reporting the highest incidence at 410 cases, and Yogyakarta

recorded the lowest at 93 instances. Furthermore, in Yogyakarta, 12 individuals were recorded with DHF cases which resulted in death, with the highest number coming from Kulon Progo district, accounting for 6 individuals (Dinkes DIY, 2021).

Dengue has the potential to reduce life expectancy (Martelli *et al.,* 2011). An individual's quality of life may be affected by their level of knowledge (Prasetyani, 2015). One of the generic instruments for measuring quality of life is the EuroQOL-5 Dimension (EQ-5D). This measurement instrument is a general instrument that is widely used in measuring health status in a population. The quality of life of patients after receiving treatment can be measured in 5 areas, including: 1) mobility; 2) self-care; 3) typical activities; 4) discomfort/pain; 5) depression/anxiety (van Reenen *et al.,* 2019). Meanwhile, VAS (visual analog scale) measurements are useful in assessing health status on a scale of 100 mm with scores ranging from 0 (indivative of very poor health level/equivalent to death) to 100 (representing excellent health) (van Reenen *et al.,* 2015).

Medical costs can predict the costs caused by one disease in a population (Kemenkes RI, 2013). Previous research from 2015, encompassing data from three provinces—Jakarta, Bali, and Yogyakarta—indicated that dengue fever imposed an economic impact of 381,150,000 USD (Nadjib, 2019). Another study conducted at Condong Catur Hospital, Yogyakarta in 2019 showed that the cost of treatment for dengue patients refers to the clinical pathway of IDR 119,127,000.00 (Rohman & Susilowati, 2020). The rise in dengue incidence affects the financial burden of medical expenses incurred by both individuals and the government, which can be assessed through the treatment burden that includes direct costs (Halasa *et al*., 2012).

The aim of this research was to find out the level of quality of life and cost of treatment for dengue patients when hospitalized at the two hospitals that were the research locations.

2. Method

2.1. Research design

The current study employed a cross-sectional design from January to August 2023. It was conducted at two PKU Muhammadiyah hospitals in Yogyakarta and Bantul. The research ethics committee of PKU Muhammadiyah Hospital Yogyakarta has provided a statement of ethical feasibility for this study through letter number Ref.: 00069/KT.7.4/III/2023. We also obtained a research permit from PKU Muhammadiyah Hospital in Bantul through letter number: 2554/P.24.2/VIII/2022 dan 010/KET/B/03.23.

2.2 Sampling and data collection techniques

Respondents were selected based on the study's objective and design. Respondents included patients diagnosed with expanded dengue syndrome (DSS), dengue hemorrhagic fever (DHF), and dengue fever. The respondents consisted of patients of all age groups who were hospitalized at the specified hospitals between January and August 2023. The patients should consent to participate in the study by signing an informed consent. The exclusion criteria for this study included patients suspected or confirmed to have COVID-19, those with comorbidities, individuals referred to other hospitals, and patients who had died.

Data collected in this study included patient characteristics, including gender, age, payment status, and length of hospital stay. Quality of life of pediatric patients was determined using the EQ-5D-Y questionnaire, while quality of life of adult patients was determined using the EQ-5D-5L questionnaire. Prior to their use, the questionnaire items had been translated into Indonesian and validated with an r-value of 0.718 (Sari *et al*., 2015). Quality of life was measured through five dimensions, including walking ability, daily activities, self-care, discomfort/pain, and sadness/depression/anxiety. Respondents' health scores were measured through their perceptions using the VAS scale. Data on medical expenses would refer to direct medical costs obtained from the database in the finance unit at both hospitals. Medical expenses included the cost of nursing and doctor services, inpatient costs, and pharmaceuticals.

2.3 Data analysis

The data were then analyzed descriptively and presented as a percentage of quality of life and average medical costs.

3. Results and discussion

Patients with dengue who were hospitalized throughout January-August 2023 at the study locations amounted to 20 patients. These patients were selected according to the inclusion and exclusion criteria. Details of dengue patients who matched the characteristics of the study are presented in **Table 1**.

Table 1 shows that dengue can attack individuals of all age groups. The largest number of DHF cases were found among children and adolescents. At the PKU Muhammadiyah Hospital in Bantul, the number of children aged 1-15 years infected with dengue was 57.1%. Meanwhile, at the PKU Muhammadiyah Hospital in Yogyakarta City, 53.8% of adolescents aged 16-25 years

were infected with dengue. This data pertains to the findings of a study by Ratnawiningsih *et al.* (2022) which stated that the patients most infected with dengue were adolescents and children. The child's underdeveloped immune system is the explanation for the increased susceptibility to dengue infection in children. Meanwhile, in cases of dengue infecting adolescents, this is comparable to the study (Islammia *et al.,* 2022), which stated that 32% of dengue patients were adolescents aged 17-25 years. This study found that adolescents possess an elevated risk of *Aedes aegypti* mosquito bites due to their frequent engagement in outdoor activities.

Table 1. Characteristics of dengue patients in both hospitals in january-august 2023

Characteristics	Number of patients					
	PKU Muhammadiyah Yogyakarta n=13(%)			PKU Muhammadiyah Bantul n=7(%)		
	DF	DHF	DSS	DF	DHF	DSS
Age group						
1-15 years old	3(23.1)	-	-	4(57.1)	-	-
16-25 years old	6(46.2)	1(7.7)	-	1(14.3)	-	-
26-59 years old	1(7.7)	1(7.7)	-	-	1(14.3)	-
>60 years old	-	1(7.7)	-	1(14.3)	-	-
Gender						
Female	5(38.5)	1(7.7)	-	3(42.9)	-	-
Male	5(38.5)	2(15.4)	-	3(42.9)	1(14.3)	-
Occupation						
Working	4(30.8)	2(15.4)	-	2(28.6)	1(14.3)	
Not working	6(46.2)	1(7.7)	-	4(57.1)	-	
Education						
No education	1(7.7)	-	-	1(14.3)	-	-
Elementary school	2(15.4)	-	-	3(42.9)	-	-
Junior high school	-	1(7.7)	-	-	-	-
Senior high school	4(30.8)	1(7.7)	-	1(14.3)	1(14.3)	-
University	3(23.1)	1(7.7)	-	1(14.3)	-	-
Payment Status						
BPJS (national insurance)	8(61.5)	2(15.4)	-	6(85.7)	1(14.3)	-
No insurance	2(15.4)	1(7.7)	-	-	-	-
Length of hospital stay						
Average length = 4 days	1(7.7)	2(15.4)	-	4(57.1)	-	-
Average length ≤ 4 days	5(38.5)	1(7.7)	-	2(28.6)	-	-
Average length >4 days	4(30.8)	-	-	-	1(14.3)	-

The majority of dengue cases in both hospitals were male, with 7 and 4 individuals, respectively. These data illustrate that men have a higher risk of dengue infection compared to

women. Sihite's research also states that dengue is more likely to infect men (68.24%), because men tend to have higher activities and mobility outside the home than women (Sihite *et al.,* 2017). This finding is also in accordance with data from the Indonesian Ministry of Health, indicating that the dengue virus infects more men (53.08%) than women (Kemenkes RI, 2021).

Based on employment status, the dengue virus mostly infects unemployed people, namely 11 patients from both hospitals. The data found is relevant to the research of Ramadani *et al.* (2023) which indicated that unemployed dengue sufferers were 62.9%. The reason is that most dengue patients are students and elderly who are no longer economically active, typically engaging in activities centered around their homes. This condition affects their inadequate knowledge regarding dengue prevention. Because the transmission and spread of dengue disease occurs evenly in the same environment, these unemployed people are susceptible to being infected due to limited information. Dengue disease transmission occurs in all populations. High number of cases in neighborhoods with high population and high mobility (Carrington & Simmons, 2014).

Dengue patients at PKU Muhammadiyah Hospital, Yogyakarta City were mostly dominated by high school graduates (38.5%). This result is relevant to previous research which found that education level is usually related to the diversity of activities and busyness that reduce body immunity (Ramadani *et al.,* 2023). This finding is also related to the research results of Dwi *et al*. (2011) which revealed that most infected patients had a high school education level, totaling 84 people (41%).

The payment status of dengue patients in both hospitals is divided into two methods, namely *BPJS* (insurance) and general (without insurance). In this study, most patients (76.9% and 100% respectively) utlized insurance or using *BPJS* services for payment. Payment via *BPJS* is an indication of increased public awareness of utilizing national health insurance from the government. This is in accordance with previous research at PKU Muhammadiyah Bantul Hospital in 2022, suggesting that patients whose payment status utilized government insurance (BPJS) had a higher percentage, namely 82.5% compared to patients who paid for hospital services without insurance (*BPJS*) (Ratnawiningsih *et al.,* 2022).

The shortest length of hospital stay for patients was 2 days and the longest was 9 days. From the findings of this study, dengue patients spent less than or equal to 4 days in each hospital. The findings align with the results of Amini's study which revealed that the length of

hospitalization of dengue patients was ≤ 4 days (77.3%) (Amini *et al.,* 2019). Length of hospitalization the most <6 days 67 patients (71%), and the most discharged conditions were 93 patients (99%) recovered (Islammia *et al*, 2022).

The quality of life of child respondents aged 1-15 years was assessed using the EQ-5D-Y questionnaire. Meanwhile, the quality of life of adolescent and adult patients aged 16 years or older was assessed using the EQ-5D-5L questionnaire. The results of this study are shown through two aspects, namely VAS (health score) and health quality. The health profile assessment of respondents was carried out through five dimensions, namely walking ability, routine activities, self-care, discomfort/pain, and depression/anxiety/sadness. The results of the study regarding the quality of life of respondents can be seen in **Table 2** and **Table 3**.

According to **Table 2**, the walking ability of pediatric patients in both hospitals is at level 1. In other words, 100% of the children patients exhibited no issues in walking. However, 66.7% of adult DHF patients at PKU Muhammadiyah Hospital, Yogyakarta, experienced difficulties with walking, and 50% of adult DHF patients at PKU Muhammadiyah Hospital Bantul also faced similar challenges.

Table 2. Quality of life of pediatric DHF patients in both hospitals from January to August 2023

Dimension		Inpatient Aged 1 –16 years old					
		PKU Muhammadiyah Yogyakarta n= 3 (%)			PKU Muhammadiyah Bantul n=4 (%)		
		DF n=3	DHF n=0	DSS n=0	DF n=4	DHF n=0	DSS n=0
Walking ability	Level 1	100	-	-	100	-	-
	Level 2	-	-	-	-	-	-
	Level 3	-	-	-	-		
Self-care	Level 1	-	-	-	-	-	-
	Level 2	100	-	-	50		
	Level 3	-	-	-	50		
Routines	Level 1	-	-	-	75	-	-
	Level 2	100	-	-	-		
	Level 3	-			25		
Pain/discomfort	Level 1	100	-	-	25	-	-
	Level 2	-	-	-	75		
	Level 3	-	-	-	-		
Anxiety/depression/ sadness	Level 1	-	-	-	25	-	-
	Level 2	100	-	-	75		
	Level 3	-	-	-	-		
VAS score (Mean ± SD)		72.33 ± 10.78	-	-	74.25 ± 6.50	-	-

Notes: Level 1 = 1 (no issue); Level 2 = 2 (few issues); Level 3 = 3 (many issues)

All pediatric DF patients at PKU Muhammadiyah Hospital in Yogyakarta city experienced slight problems in performing self-care, while only a portion of pediatric DF patients at PKU Muhammadiyah Hospital in Bantul experienced difficulties in performing self-care. Meanwhile, only a few adult DF patients at PKU Muhammadiyah Hospital in Yogyakarta city (14.3%) experienced many problems (level 5) in performing self-care. In addition, 50% of adult DHF patients at PKU Muhammadiyah Hospital in Bantul experienced many problems in performing self-care. According to research data, a quarter (25%) of pediatric DF patients at Muhammadiyah Hospital in Bantul felt they had many problems in carrying out routines. Meanwhile, 71.4% of adult DF patients at PKU Muhammadiyah Hospital in Yogyakarta city experienced problems in carrying out routines, 50% of DF patients and 100% of adult DHF patients in Bantul experienced few issues in carrying out routines.

A few (14.3%) adult DF patients at PKU Muhammadiyah Hospital, Yogyakarta felt discomfort or pain at level 3, 4, and 5. Pain/discomfort at level 2 was observed among the majority (57.1%) of adult DF patients at PKU Muhammadiyah Hospital, Yogyakarta. Most pediatric DF patients (75%) at PKU Muhammadiyah Hospital, Bantul reported discomfort at level 2. Discomfort at level 3 was found in adult DHF patients at both hospitals with percentages of 66.7% and 100%, respectively. A small proportion (14.3%) of adult DF patients at PKU Muhammadiyah Hospital, Yogyakarta reported anxiety/depression at level 5. Pediatric DF patients at both hospitals experienced anxiety/depression/sadness at level 2 at both hospitals with percentages of 100% and 75%, respectively. Anxiety/depression/sadness at level 3 was also observed among 28.6% of adult DF patients at PKU Muhammadiyah Hospital, Yogyakarta and 50% of DF patients at PKU Muhammadiyah Hospital in Bantul. In addition, 100% of adult DHF patients in Bantul stated that they experienced depression/anxiety/sadness at level 3.

Child respondents from PKU Muhammadiyah Hospital in Yogyakarta reported a mean VAS Score of 72.33±10.78, lower than that found in child patients in Bantul with a mean score of 74.25±6.50. On the other hand, adult DF patients from PKU Muhammadiyah Hospital in Yogyakarta showed a mean VAS score of 67.14±14.39, higher than that observed among the DHF adult patients from Bantul with a mean VAS score of 63.33±7.63. Meanwhile, the DF adult patients from PKU Muhammadiyah Hospital in Bantul indicated a mean VAS score of 65.00 ± 7.07, lower than that reported by their DHF patients with a mean VAS score of 80.00 ± 0.00. This finding may be due to several factors that enhance quality of life, including nutritional fulfillment and nursing care patterns (Sumaryati *et al.*, 2019). The results of this study indicate

that dengue patients, both at PKU Muhammadiyah Hospital in Bantul and Yogyakarta, have good health scores. These results are in line with previous research at PKU Muhamadiyah Hospital Bantul which revealed that the Hospital has a good health score (Ratnawiningsih *et al.,* 2022).

Table 3. Quality of life of adult DHF patients in both hospitals from January to August 2023

Dimension		Inpatient Aged ≥17 – ≥65 years old					
		PKU Muhammadiyah Kota Yogyakarta n=10(%)			PKU Muhammadiyah Bantul n=3(%)		
		DF n=7	DHF n=3	DSS n=0	DF n=2	DHF n=1	DSS n=0
Walking ability	Level 1	42.9	-	-	50	100	-
	Level 2	42.9	33.3	-	-	-	-
	Level 3	14.3	66.7	-	50	-	-
	Level 4	-	-	-	-	-	-
	Level 5	-	-	-	-	-	-
Self-care	Level 1	-	-	-	-	-	-
	Level 2	42.9	66.7	-	50	100	-
	Level 3	14.3	-	-	-	-	-
	Level 4	28.6	33.3	-	50	-	-
	Level 5	14.3	-	-	-	-	-
Routines	Level 1	14.3	-	-	-	-	-
	Level 2	71.4	66.7	-	50	-	-
	Level 3	-	33.3	-	50	100	-
	Level 4	-	-	-	-	-	-
	Level 5	14.3	-	-	-	-	-
Pain/discomfort	Level 1	-	-	-	-	-	-
	Level 2	57.1	33.3	-	50	-	-
	Level 3	14.3	66.7	-	-	100	-
	Level 4	14.3	-	-	50	-	-
	Level 5	14.3	-	-	-	-	-
Anxiety/depression/ sadness	Level 1	28.6	66.7	-	50	-	-
	Level 2	28.6	33.3	-	-	-	-
	Level 3	28.6	-	-	50	100	-
	Level 4	-	-	-	-	-	-
	Level 5	14.3	-	-	-	-	-
VAS Score (Mean ± SD)		67.14 ± 14.39	63.33 ± 7.63	-	65.00 ± 7.07	80.00 ± 0.00	-

Notes: Level 1 = 1 (no issue); Level 2 = 2 (few issues); Level 3 = 3 (some issues); Level 4 = 4 (many issues); Level 5 = 5 (a lot of issues)

The medical costs collected in this study were direct medical costs in the form of pharmaceutical costs (medicines and medical devices), doctor's services, nurse's services, pharmacist's services, nutritionist's services, laboratory costs, inpatient costs, and administrative costs. More complete data related to the medical costs of dengue patients who

were hospitalized at PKU Muhammadiyah Hospital in Yogyakarta can be seen in **Table 4**, while complete data related to the medical costs incurred by dengue patients at PKU Bantul Hospital can be seen in **Table 5**.

According to **Table 4**, dengue medical costs were paid by two methods, namely general payment (without insurance) and payments with insurance (*BPJS*). **Table 4** contains the total medical costs of DF patients which were paid without insurance. The highest inpatient cost for patients without insurance was IDR 2,175,000 (patients hospitalized in a VIP room). On the other hand, the highest inpatient cost for DF patients with *BPJS* insurance (class III-inpatient room) was IDR 1,556,873 for laboratory costs.

Table 4. Dengue medical costs at PKU Muhammadiyah Hospital in Yogyakarta from January to August 2023

Cost component	Class	Medical costs (IDR)					
		No insurance (n=3)			*BPJS* (n=10)		
		DF n=2	DHF n=1	DSS n=0	DF n=8	DHF n=2	DSS n=0
Pharmaceutical cost	VIP	722,950	1,856,500	-	-	-	-
	I	-	-	-	333,650	-	-
	II	-	-	-	235,650	-	-
	III	-	-	-	481,774	274,250	-
Treatment cost	VIP	1,154,250	1,550,000	-	-	-	-
	I	-	-	-	931,313	-	-
	II	-	-	-	647,000	-	-
	III	-	-	-	848,188	628,625	-
Laboratory cost	VIP	729,250	1,017,000	-	-	-	-
	I	-	-	-	502,245	-	-
	II	-	-	-	595,745	-	-
	III	-	-	-	1,556,873	1,294,995	-
Inpatient cost	VIP	2,175,000	2,400,000	-	-	-	-
	I	-	-	-	600,000	-	-
	II	-	-	-	450,000	-	-
	III	-	-	-	685,000	540,000	-
Administrative cost	VIP	138,000	158,000	-	-	-	-
	I	-	-	-	122,500	-	-
	II	-	-	-	88,750	-	-
	III	-	-	-	86,500	81,500	-
Total medical cost	VIP	4,919,450	6,981,500	-	-	-	-
	I	-	-	-	2,489,708	-	-
	II	-	-	-	2,017,145	-	-
	III	-	-	-	3,659,585	2,819,370	-
Average		**4,919,450**	**6,981,500**	**-**	**2,722,146**	**2,891,370**	**-**

The highest average medical expenses paid by DHF patients without insurance were IDR 2,400,000 (VIP class), while the highest cost paid by DHF patients with BPJS insurance was of laboratory

cost, which was IDR 1,294,995 (class III). Based on the information collected, the National Health Insurance covers approximately one-quarter of the medical expenses of dengue patients (for the national scale it is 25%; for Yogyakarta 26%). In 2017, the medical expenses paid by the JKN company (for BPJS insurance) were USD 95.03 USD nationally and USD 0.386 in the Yogyakarta city area (Wilastonegoro *et al*., 2020).

Table 5. Dengue medical costs at PKU Muhammadiyah Hospital in Bantul from January to August 2023

Cost component	Class	Medical costs (IDR)					
		No insurance n=0			*BPJS* n=7		
		DF n=0	DHF n=0	DSS n=0	DF n=6	DHF n=1	DSS n=0
Pharmaceutical cost	VIP	-	-	-	697,500	-	-
	I	-	-	-	510,000	-	-
	II	-	-	-	215,000	-	-
	III	-	-	-	320,650	997,700	-
Treatment cost	VIP	-	-	-	1,048,000	-	-
	I	-	-	-	796,500	-	-
	II	-	-	-	853,500	-	-
	III	-	-	-	858,000	962,500	-
Laboratory cost	VIP	-	-	-	732,833	-	-
	I	-	-	-	750,000	-	-
	II	-	-	-	198,000	-	-
	III	-	-	-	698,000	876,500	-
Inpatient cost	VIP	-	-	-	1,343,333	-	-
	I	-	-	-	700,000	-	-
	II	-	-	-	500,000	-	-
	III	-	-	-	315,000	1,800,000	-
Administrative cost	VIP	-	-	-	132,667	-	-
	I	-	-	-	80,000	-	-
	II	-	-	-	76,000	-	-
	III	-	-	-	80,000	161,000	-
Total medical cost	VIP	-	-	-	3,954,332	-	-
	I	-	-	-	2,836,500	-	-
	II	-	-	-	1,842,500	-	-
	III	-	-	-	2,271,650	4,797,700	-
Average medical costs		**-**	**-**	**-**	**2,726,245.5**	**4,797,700**	**-**

Table 5 suggests that patients at PKU Muhammadiyah Hospital in Bantul mostly utilized BPJS insurance payments. The highest medical cost observed among DHF patients who used BPJS insurance services for class I, II, III, and VIP was IDR 4,797,700, while among DF patients it was IDR 3,954,332. The average medical costs incurred by DF and DHF patients at PKU Muhammadiyah Hospital in Yogyakarta using BPJS insurance were IDR 2,722,146 and IDR 2,891,370. On the other hand, the average medical

costs paid by DF and DHF patients at PKU Muhammadiyah Hospital Bantul were IDR 2,726,245 and IDR 4,797,700. The findings of the study indicate that DHF patients pay higher medical costs than DF patients, both in terms of administrative costs, pharmacy, laboratory, inpatient care, and treatment costs. In addition, DHF patient care is also longer than DF patients. This is not in line with previous studies, which showed that DSS/DSS patients have the highest medical costs because DSS/DSS patients have worse conditions than DHF patients and because DSS/DSS patients require more intensive treatment to prevent dengue infection (Ratnawiningsih *et al*., 2022). In this study, we observed no dengue patients with DSS condition, so this study was limited to patients with two types of dengue (DF and DHF).

4. Conclusion

The results showed that the majority of pediatric DF patients in both hospitals reported anxiety and depression, with percentages of 100% (Yogyakarta) and 75% (Bantul), respectively. Meanwhile, most adult DF patients in both hospitals experienced problems in carrying out routines, with percentages of 71.4% (Yogyakarta) and 50% (Bantul), respectively. More than half of adult DHF patients in both hospitals reported pain and discomfort, with percentages of 66.7% (Yogyakarta) and 100% (Bantul), respectively. The highest average medical costs paid by DF and DHF patients at PKU Yogyakarta Hospital were IDR 4,919,450 and IDR 6,981,500. These patients paid without insurance. On the other hand, DF and DHF patients at PKU Hospital chose to pay with *BPJS* insurance, with the highest average medical costs of IDR 2,726,245.5 (for DF patients) and IDR 4,797,700 (for DHF patients) to cover for laboratory costs.

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Potential advancement utilizing nanotechnology-based delivery system to enhance the therapeutic properties of lavender essential oil: a review article

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Abstract

Background: Essential oils (EO), such as lavender (Lavandula spp.), which are derived from its flower, have become a growing trend in recent years as an alternative medicine in therapy. However, the poor physicochemical properties of EOs have several disadvantages for their application. Integrating nanotechnology into the formulation of medicinal dosage forms may provide a viable way to mitigate these limitations and improve the effectiveness of EO utilization.

Objective: This literature review aims to gather information on the medicinal benefits of lavender EO and the prospective applications of nanotechnology-based delivery systems for EOs.

Method: Online databases such as PubMed, Science Direct, Research Gate, Google Scholar, and other reliable sources were used to find 65 publications between 1991 and 2021, which are assessed in this review.

Results: It is revealed that the main constituents of lavender EO are linalool, linalyl acetate, and lavandulyl acetate. Its effectiveness as an antibacterial, antioxidant, anticancer, anti-anxiety, and having effects on the central nervous system, along with other qualities including pain relief, has been demonstrated via numerous studies. Additionally, it was demonstrated to have dermatological properties that helped treat dermatitis and encouraged both hair growth and wound healing. Furthermore, it was also found that the application of nanotechnology in EOs has improved the concentration of active compounds in the blood and produced a stable dosage form while also increasing its efficacy.

Conclusion: It is feasible to create nanoparticle dosage forms from lavender EO that can improve the substance's solubility, stability, and pharmacological effects. More research is necessary to formulate lavender EO by applying nanotechnology.

Keywords: Lavender, essential oil, therapeutic activity, nanotechnology, nanoparticle

1. Introduction

Essential oils (EO) have become increasingly valuable for their potential use in medicine, cosmetics, aromatherapy, fragrance, and spirituality. Depending on the section and kind of plant, different methods are used to extract EOs from the leaves, flowers, stems, fruit, seeds, bark, and roots of a variety of aromatic plants. The unique aromas of EO can be attributed to about 3000 phytochemicals, including saturated and unsaturated hydrocarbons, terpenes, sesquiterpenes, alcohols, phenols, aldehydes, ketones, esters, acids, phenolic ethers, oxides, lactones, and coumarins. These colorless liquid oils are extremely powerful and concentrated, reviving and stimulating pressure points in addition to supporting a range of medical functions (Ali *et al.*, 2015; Dunning, 2013).

Lavender (Lavandula spp.), a beloved garden herb that has long been used as medicine, is one of the most common uses of essential oils. Lavenders are native to the mountainous regions of the Mediterranean and belong to the Labiatae (Lamiacae) family. They are utilized for a number of therapeutic and cosmetic uses. More than 30 species of plants belong to the Lamiaceae family, and

the most well-known are *Lavandula stoechas* (French lavender), *Lavandula latifolia* (wide leaves lavender), and *Lavandula angustifolia* Mill (narrow leaves lavender). Due to its unique medicinal properties, the narrow-leaved lavender (*Lavandula angustifolia*, originally *L. officinalis* Chaix or *L. vera*), also referred to as garden lavender, is the most valuable genus of lavender (Adaszyńska *et al*., 2013). Lavender EO is obtained through steam distillation of both the flower heads and the leaves; however, due to their distinct chemical compositions, the flower yields a more fragrant and sweeter oil. It is a pale yellowish liquid with a smell that varies from fresh and flowery to aromatic and woodsy. In addition to its supposed benefits for burns and bug bites, the oil is also reported to possess antibacterial, antifungal, carminative (smooth muscle relaxing), sedative, and antidepressive qualities (Cavanagh & Wilkinson, 2002; Miastkowska *et al*., 2021).

At present, EO's potential as a therapeutic agent has never completely utilized due to its lipophilic component and extreme volatile. Consequently, EOs may be impacted by conversion and degrading processes. EOs are prone to oxidation and polymerization, which can lead to degradation and changes in their pharmacological properties (Turek & Stintzing, 2013). The use of nanoparticle technology in the pharmaceutical industry can offer several benefits that can increase EO effectiveness to address these shortcomings. Currently, there have been multiple studies showcasing the applications of nanotechnology to formulate lavender EO dosage form. However, there aren't any articles that specifically review the actions and recent advancement of lavender EO. Thus, the purpose of this review is to compile extensive data regarding the activity of lavender EO as well as its possible advancement via the application of nanotechnology.

2. Method

This literature review is performed by examining article publication available online in PubMed, Science Direct, Research Gate, Google Scholar and other reliable sources via terms like “lavender essential oil”, “chemical compounds”, “therapeutic activity”, “nanotechnology”, and “nanoparticle” that were used for each of the searches. From the database, 65 articles were selected which are published national and international article between 1991 through 2021, in either English or Indonesia language.

3. Result and discussion

3.1. Chemical compounds of lavender essential oil

Study by de Groot and Schmidt (2016) analyzed lavender EO samples from France, Bulgaria, Australia, Ukraine, Moldavia, England, and China between 2001 and 2013 by GC/MS, and concluded

the following ten compounds that has the highest maximum concentrations found in the oil are as follows: linalool (26.0%-44.8%), linalyl acetate (26.1%-43.3%), (Z)-A-ocimene (0.3%-7.5%), lavandulyl acetate (0.4%-6.3%), terpinen-4-ol (0.07%-5.9%), acaryophyllene (1.8%-5.9%), (E)-A-ocimene (0.7%-4.7%), (E)-Afarnesene (0.4%-4.5%), borneol (0.3%-2.7%), and 1,8-cineole (0.01%-2.4%) (De Groot & Schmidt, 2016). Another test in 2014 also found that linalool (34.1%) and linalyl acetate (33.3%) were discovered to be the two primary ingredients of lavender oil (**Figure 1.**), while other notable constituents were ocymene and lavandulil acetate (3.2% each) (Sienkiewicz *et al.*, 2014). Other research by Vârban *et al.* (2022) using GC/MS analyzing lavender EO resulted in 38 of the 40 isolated chemicals that were identified using their mass spectra (29 terpenoids, 2 alcohols, 1 ketones and 5 esters, 1 hydrocarbon). The volatile profile was made up 94.41% by terpenes and terpenoids. Beta-linalool and linalool acetate were the two most prevalent volatile substances, followed by caryophyllene (5.66%) and beta-farnesene (6.45%), while camphor was only present in very trace amounts (0.18%). This kind of volatile character is said to be indicative of the highest-quality lavender EO (Vârban *et al.*, 2022).

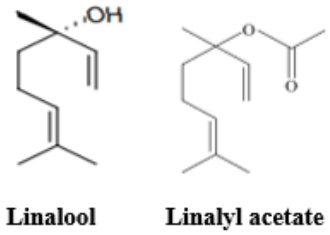

Figure 1. Two main compounds of lavender EO

3.2. Pharmacological activities

Numerous articles were assessed to obtain data regarding the potential therapeutic activity of lavender EO as shown in **Table 1**.

Table 1. Studies on lavender EO therapeutic activity

Author(s)	Year	Therapeutic activity/response	Disease
Soković *et al*.	2007	Showed antimicrobial properties at concentrations of 4.0-9.0 mg/ml	Bacterial infection
Mayaud *et al*.	2008	Effective against gram-positive bacteria more than gram-negative bacteria	Bacterial infection
Hanamanthagouda *et al*.	2010	Diverse biological actions of antibacterial and antifungal depends on the genus of Lavandula	Bacterial and fungi infection
Stanojevic *et al.*	2011	Inhibition of multiple strains of bacteria	Bacterial infection
Hui *et al*.	2010	Inhibitory effect on the processes of fat oxidation and lipid peroxidation	Free radicals
Viuda-Martos *et al*.	2011	Reduce 50% DPPH at multiple concentration of	Free radicals

Author(s)	Year	Therapeutic activity/response	Disease
		lavender EO	
Lin *et al*.	2009	Exhibit antioxidant properties akin to lime and marjoram EO in same study	Free radicals
Wattenberg	1991	Rats fed the lavender oil-containing diet had noticeably fewer tumors and adenomas per rat	Cancer
Teranishi *et al*.	1993	Containing compounds in trace amount that may have potent anticancer properties	Cancer
Zhang *et al.*	1999	Containing monoterpenoid perillyl alcohol inhibits the growth and division of angiogenic cells in vitro	Cancer
Loutrari *et al*.	2004		
Moteki *et al*.	2002	Contents of terpinen-4-ol and 1,8-cineole, have been shown to have anticancer properties in vitro by inducing apoptosis in tumor cells	Cancer
Calcabrini *et al.*	2004		
Yamada *et al*.	1994	Inhaling or injecting lavender EO intraperitoneally can prevent convulsions caused by pentylenetetrazol or nicotine	Anxiety
Dunn *et al*.	1995	Lavender EO aromatherapy was found to reduce anxiety in comparison to receiving a massage without aromatherapy and to resting	Anxiety
Kritsidima *et al*.	2010	Used of lavender essential oil aromatherapy to reduce their anxiety about the uncomfortable feelings	Anxiety
Buchbauer *et al*.	1991	Linalool and terpineol, has been shown to reduce anxiety, encourage sleep, and weaken physical activity in both humans and animals	Central nervous system
Hudson	1995	Inhaling lavender oil increased sleep quality for 72% of patients, compared to only 11% of the control group	Central nervous system
Diego *et al*.	1998	Mice and rats given systemic injections of lavender EO exhibit sleepiness. Inhaling lavender EO improved arithmetic performance and increased wave activity of forty healthy individuals in brain wave research	Central nervous system
Styles	1997	Young HIV patients who had lavender EO massages required less painkillers overall	Pain
Lis-Balchin & Hart	1999	Lavender EO has an antispasmodic effect via increasing messenger cAMP levels	Pain
Ghelardini *et al*.	1999	*In vitro* studies have confirmed this EO's analgesic activity, and anaesthetic properties have been proven in rabbit experiments	Pain
Anderson *et al*.	2000	As an alternative of topical steroids to treat eczema, lavender EO showed substantial reduction in irritability and sleeping disruption	Eczema
Kim & Cho	2010	Using lavender oil topically may be able to block part of the allergic pathway	Skin allergic reaction
Koca Kutlu *et al*.	2013	Higher levels of both EGF (epidermal growth factor) and FGF (fibroblast growth factor)-2 in wounds treated with lavender EO	Wound
Mori *et al*.	2016	Lavender EO was found to significantly raise the levels of type I collagen and TGF-β (transforming growth factor)-β	Wound

Author(s)	Year	Therapeutic activity/response	Disease
Hay *et al*.	1998	Combination of oil containing thyme, rosemary, cedarwood and lavender EO showed hair growth improvement in randomized controlled study of alopecia areata patients	Alopecia
Lee *et al*.	2016	Lavender oil significantly promotes hair development, as seen both morphologically and histologically in female C57BL/6 mice	Alopecia

3.2.1. Antimicrobial

At concentrations of 4.0–9.0 mg/ml, the lavender EO (*L. angustifolia*) exhibited antimicrobial properties (Soković *et al.*, 2007). Its antibacterial activities at doses of 0.94%–10% against 65 bacterial species were proven in the investigation by Mayaud *et al*., (the effectiveness against Gram-positive bacteria was higher than against Gram-negative) (Mayaud *et al.*, 2008). The growth of *S. enteritidis, K. pneumoniae, E. coli, S. aureus, P. aeruginosa, C. albicans,* and *A. niger* are all inhibited by lavender EO (Stanojevic *et al*., 2011). The biological actions of the EOs produced by plants of the genus Lavandula are extremely diverse. The growth of germs like Salmonella, Enterobacter, Klebsiella, *E. coli, S. aureus*, and *L. monocytogenes* are inhibited by the EO of *Lavandula dentata*. At concentrations of 0.5-2.0 µg×ml-1 for bacteria and 2.0-4.0 µg×ml-1 for fungi, respectively, the EO of *L. bipinnata* demonstrates antibacterial activity (against *E. coli, P. aeruginosa, S. aureus, and B. subtilis*) and antifungal properties (against *A. niger, P. notatum,* and *C. albicans*) (Hanamanthagouda *et al*., 2010).

3.2.2. Antioxidant

The antioxidant qualities of lavender EO protect cells from the damaging effects of free radicals. In a linoleic acid model system, Hui *et al.* (2010)'s research demonstrated this oil's inhibitory effect on the processes of fat oxidation and lipid peroxidation (Hui *et al.*, 2010). Studies examining this EO's ability to reduce 50% DPPH radicals yielded inconsistent findings; values ranged from 289 µg×ml-1 to 48.7 mg×ml-1 (Viuda-Martos *et al*., 2011). Another study used DPPH to examine the antioxidant properties of lavender essential oil (*L. angustifolia*) and how well it can counteract free radicals. At a concentration of 5 g×1-1, the reading of 15.18 ± 0.009% indicates properties akin to those of lime and marjoram essential oils (Lin *et al*., 2009). They discovered that the EO had a far lesser ability to neutralize free radicals at a comparable quantity (4.11%).

3.2.3. Anticancer

To explore the anticancer potential of lavender oil components, numerous in vitro research has been conducted using rat, bovine, human, and bacterial cell lines. These investigations have shown that lavender oils contain compounds that, in trace amounts, may have potent anticancer properties (Teranishi *et al*., 1993). Many lavender essential oils include nerolidol, a sesquiterpene

that is thought to have anticancer effects since rats fed the oil-containing diet had noticeably fewer tumors and adenomas per rat (Wattenberg, 1991). It also has been demonstrated that the monoterpenoid perillyl alcohol inhibits the growth and division of angiogenic cells *in vitro*, which makes it a viable candidate for use in anticancer treatment (Loutrari *et al.*, 2004; Zhang *et al*., 1999). Later, perillyl alcohol's potential as a chemo preventive agent was explored through National Cancer Institute-sponsored Phase I, II, and III trials for colon, breast, and prostate cancers. Some constituents of lavender oil, such as terpinen-4-ol and 1,8-cineole, have been shown to have anticancer properties *in vitro* by inducing apoptosis in tumor cells (Calcabrini *et al*., 2004; Moteki *et al*., 2002).

3.2.4. Anti-anxiety

In a randomized clinical experiment with 122 critically ill patients, EO aromatherapy was found to reduce anxiety in comparison to receiving a massage without aromatherapy and to resting. Blood pressure and respiratory system health did not differ between the two groups (Dunn *et al*., 1995). According to other research, inhaling or injecting lavender EO intraperitoneally can prevent convulsions caused by pentylenetetrazol or nicotine (Yamada *et al*., 1994). Another study on people waiting at dentist clinics used lavender essential oil aromatherapy to reduce their anxiety about the uncomfortable feelings they expected (Kritsidima *et al.*, 2010).

3.2.5. Central nervous system

A study by Buchbauer *et al.* (1991) found that specific EO components, including linalool and terpineol, have an impact on the central nervous system. This has been shown to reduce anxiety, encourage sleep, and weaken physical activity in both humans and animals (Buchbauer *et al.*, 1991). A 245-person clinical experiment found that inhaling lavender oil increased sleep quality for 72% of patients, compared to only 11% of the control group. Four out of every five patients who took the medicine reported feeling generally well, compared with only 25% of individuals in the control group (Hudson, 1995). Mice and rats given systemic injections of lavender essential oil exhibit sleepiness. Inhaling lavender essential oil (EO) improved arithmetic performance and increased wave activity of forty healthy individuals in brain wave research. In addition to being sleepy, patients have also allegedly reported feeling relaxed and having a positive attitude on life (Diego *et al*., 1998).

3.2.6. Pain relieves

Young HIV patients who had lavender essential oil massages required less painkillers overall and, in certain cases, felt no discomfort at all (Styles, 1997). Lavender essential oil (EO) has an antispasmodic effect via increasing messenger cAMP levels, while the exact mechanism is unclear (Lis-Balchin & Hart, 1999). *In vitro* studies have confirmed this EO's analgesic activity, and anaesthetic properties have been proven in rabbit experiments. Anaesthetic activity was measured

using both *in vivo* testing on the rabbit conjunctival reflex and *in vitro* testing on a rat phrenic nerve-hemidiaphragm preparation. The *L. angustifolia* EO, linalyl acetate, and linalool (0.01 - 10 g/ml) substantially and dose-dependently decreased the electrically induced contractions of the rat phrenic-hemidiaphragm. In the rabbit conjunctival reflex test, the addition of 30–2500 g/ml of linalyl acetate and linalool to solution of *L. angustifolia* EO increases the number of shocks needed to induce the response in a dose-dependent manner, confirming in vivo the local anaesthetic effect found in vitro (Ghelardini *et al.*, 1999).

3.2.7. Dermatological activities

Lavender and other essential oils have been evaluated for their efficacy as an alternative of conventional drugs, like topical steroids, which are often found to be ineffective. The usefulness of several EOs, including lavender, in the treatment of eczema was investigated by Anderson *et al.* (2000) by employing both massage with the oils and the addition of the oils (6 drops of a blend of three oils in a 1:1:1 ratio) to bath water. The 8-week course of treatment led to a substantial reduction in irritability and sleeping disruption in both the massage alone and EO groups, despite the small sample size of 16 children (Anderson *et al.*, 2000). Furthermore, it has been suggested that using lavender oil topically may be able to block part of the allergic pathway (Kim & Cho, 2010).

3.2.8. Wound healing

Lavender essential oil was found to significantly raise the levels of type I collagen and TGF-β (transforming growth factor)-β in one investigation. This result supports the clinical observation of faster and greater wound contraction in the lavender-treated group as compared to the control group because TGF-β is known to induce fibroblast proliferation and differentiation into myofibroblasts, which are crucial for wound contraction via tissue shrinkage (Mori *et al.,* 2016). Another study discovered considerably higher levels of both EGF (epidermal growth factor) and FGF (fibroblast growth factor)-2 in wounds treated with lavender EO (Koca Kutlu *et al*., 2013). As its name would suggest, FGF-2 is crucial for the proliferation of fibroblasts. EGF is being researched as a possible topical treatment for chronic wounds since it is a signalling molecule that promotes fibroblast and epithelial cell migration, which causes wound contraction and epithelialization (Bodnar, 2013).

3.2.9. Hair growth

It is claimed that thyme, rosemary, cedarwood, and lavender EO promote hair growth in alopecia individuals. A randomized, double-blind, controlled study was conducted by Hay *et al.* (1998) to look at this effect, where 86 alopecia areata patients had their scalps massaged nightly with a combination of oil containing 114 mg of *Rosmarinus officinalis*, 94 mg of *Cedrus atlantica*, 108 mg of *Thyme vulgaris*, and 108 mg of *L. angustifolia*. Hair growth was assessed at 3- and 7-month

intervals. When EOs were administered, 19 out of 35 patients reported an improvement in hair growth, compared to just 6 out of 28 controls. This indicates that the treated group's area of alopecia was much smaller than that of the control group (Hay *et al*., 1998). Another study was conducted to investigate the effects of lavender oil (LO) on hair growth in female C57BL/6 mice. In comparison to the normal group (saline solution), the experimental group that received lavender oil at concentrations of 3% and 5%, respectively, had considerably more hair follicles, deeper hair follicles, thicker dermal layer, and fewer mast cells. These findings demonstrated that LO significantly promotes hair development, as seen both morphologically and histologically (Lee *et al*., 2016).

3.3. Application of nanotechnology on essential oil

Due to their traditional uses, plant-based products such as EO have several disadvantages when used as medications, including reduced solubility and bioavailability of the ingredient, increased dosage requirements, volatile and low stability, as well as prone to oxidation and polymerization (Mukherjee, 2015; Turek & Stintzing, 2013). These drawbacks can be addressed and enhanced using nanotechnology to produce better herbal dosage forms, such as liposomes, gold nanoparticles, and self-nano emulsifying drug delivery systems (SNEDDS). Active compounds' solubility, stability, and bioavailability can all be enhanced by formulations based on nanoparticles. Nanoparticle sizes can vary from 20 to 200 nm, depending upon its components and the method of production. Particle size reduction will increase a preparation's surface area and facilitate the entry of active components into hard-to-reach action locations, such as the blood-brain barrier, and their passage across cell and other membranes to the target of action. The creation of a more intricate drug delivery system has significantly improved thanks to the application of nanotechnology (Alexander *et al*., 2016; Harwansh *et al*., 2011b, 2011a; Porter *et al*., 2008). Recent studies have been conducted to assess the possibility of nanotechnology applications in formulating lavender EO into a more potent dosage form, as shown in **Table 2**.

Table 2. Recent examples of nanotechnology applications in lavender EO formulation

Author(s)	Year	Formulation	Therapeutical uses
Sofi *et al*.	2019	Composite electrospun wound-dressing nanofibers made of polyurethane containing lavender oil and silver nanoparticles	Antibacterial/Wound healing
Fadel *et al*.	2023	Nano system made of gold nanoparticles and nano-lavender EO using ultrasonic nanoemulsifying techniques	Antibacterial/Wound healing
Rahayu *et al*.	2024	Lavender EO-loaded polyurethane nanoparticles via a facile swelling-diffusion method as hydrocolloid agents	Antibacterial/Wound healing

Author(s)	Year	Formulation	Therapeutical uses
Fahimnia *et al*.	2024	Topical gel containing lavender oil loaded solid lipid nanoparticles	Antibacterial/Anti-inflammatory
Sanei-Dehkordi *et al*.	2023	Lavender and Geranium EO-Loaded nanogels using nanoemulsion-based gel method	Antibacterial/Repellent

A study employed a unique approach to create composite electrospun wound-dressing nanofibers made of polyurethane containing lavender oil and silver (Ag) nanoparticles (NP). The Ag NPs and lavender oil enhanced the hydrophilicity of the nanofibers and facilitated the proliferation of chicken embryo fibroblasts grown *in vitro* on these fiber dressings. The antibacterial efficacy of the nanofiber dressings was assessed using *E. coli* and *S. aureus*, resulting in inhibition zones of 16.2 ± 0.8 mm and 5.9 ± 0.5 mm, respectively, demonstrating the dressings' superior bactericidal capabilities (Sofi *et al.*, 2019).

In order to aid in wound healing and fight bacterial infection, another current study sought to create a unique nano system made of gold nanoparticles and nano-lavender EO using ultrasonic nanoemulsifying techniques. Unlike lavender EO, the newly created nano-gold/nano-lavender completely eradicated the microbial cells by penetrating the generated *P. mirabilis biofilm*. The MIC and MBEC (minimum biofilm eradication concentration) values were 8 and 16 µg/mL, respectively. Higher results, compared to lavender EO, were obtained when the cytotoxic effect of the innovative nano-gold and nano-lavender formulas was evaluated against WI-38 fibroblasts Vero (normal) cells (IC_{50} = 0.529 and 0.209 mg/mL, respectively). With a 96.78% wound closure rate, the nano-gold/nano-*Lavandula angustifolia* mixture demonstrated potent wound healing ability (Fadel *et al.*, 2023).

Other study also sought to formulate wound healing applications where the hydrocolloid-based preformed polyurethane (PU) NPs were used as a template for the simple swelling-diffusion of lavender EO. PU hydrocolloid was carefully combined with the Lavender EO phase that had been dissolved in an ethanol/water mixture at various volume ratios. Lavender EO diffused and became trapped in the PU NPs as a result of the high miscibility between Lavender EO and the PU matrix. Using ethanol/water at a ratio of 0/100, stable lavender EO-loaded PU NPs with high 59.67% entrapment efficiency 27.98% loading capacity were created. In phosphate buffer at pH 8.5, the release behaviour of lavender EO from the lavender EO-loaded PU NPs demonstrated sustained release for 192 hours. The biocompatibility of the NPs was demonstrated by the high cell survival of around 80% in the cytotoxicity assay employing fibroblast cells at the maximum dose of 1 mg/ml. A

transparent soft film formed from the encapsulated PU NPs demonstrated antibacterial efficacy against *E. coli* and *S. aureus* (Rahayu *et al.*, 2024).

A separate investigation involved the preparation of lavender oil-loaded Solid Lipid Nanoparticles (Lav-SLN) utilizing cholesterol and lecithin as natural lipids, followed by the characterization of the generated SLNs. Next, Carbopol 940 was used to create a topical gel (Lav-SLN-G) that contained 3% SLN. The antibacterial properties of Lav-SLN and Lav-SLN-G against *S. aureus* were evaluated. Lav-SLNs showed an % entrapment efficiency of 75.46%, a zeta potential of -21.6 mv, and a particle size of 19.24 nm. The pH and textural characteristics of the topical gel formulation were satisfactory. Lavender oil, Lav-SLN, and Lav-SLN-G had the following minimum inhibitory/bactericidal concentrations (MIC/MBC) against *S. aureus*: 0.12 and 0.24 $mgml^{-1}$, 0.05 and 0.19 $mgml^{-1}$, and 0.045 and 0.09 $mgml^{-1}$, respectively (Fahimnia *et al*., 2024).

Different study attempted to create lavender and geranium (*Pelargonium graveolens* L'Hér.) EO nanogels (NGs) with potential antibacterial and repellent properties. A nanoemulsion-based gel method was used to formulate the NGs; the zeta potentials and nanoemulsion droplet sizes were found to be 146 ± 7 and 106 ± 6 nm and −23.2 ± 0.7 and −17.4 ± 1 mV, respectively. The successful loading of EOs in NGs was validated by the ATR-FTIR analysis. *Anopheles stephensi* Liston mosquitoes were employed in repellent bioassays, which showed that geranium NG (140 min) was just as efficient as the commonly used repellent DEET (140 min). According to antibacterial testing, the nanogels successfully inhibited the development of bacteria; against *E. coli* Migula, the geranium NG demonstrated a reduction of over 90%. Higher effectiveness against *S. aureus* Rosenbach was demonstrated by the lavender NG (Sanei-Dehkordi *et al.*, 2023).

The findings of the studies mentioned above imply that lavender EOs could be developed into nanoparticle medication delivery systems. This goes to show that other therapeutic properties of lavender EO could also be beneficial from the application of nanotechnology in future studies, to improve its efficacy and stability as a pharmaceutical dosage form.

4. Conclusion

Lavender (*Lavandula angustifolia*), one of the most popular garden herbs in the world, contains EO with major constituents like linalool and linalyl acetate. Antimicrobial, antioxidant, anticancer, anti-anxiety, central nervous system, pain-relieving, dermatological, wound-healing, and hair-growth qualities are only a few of its many medical benefits. Recent research has focused on using nanotechnology to increase the effectiveness of lavender EO. Future research is needed to

create drug delivery methods using nanoparticles for the numerous medicinal advantages of lavender essential oil.

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The potential of coconut oil as an anti-obesity agent: a scoping review

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Abstract

Background: The rising global prevalence of obesity and its related health issues make it crucial to explore all potential therapeutic options. Coconut oil (CNO) has been extensively studied for its health benefits, yet no scoping review has specifically assessed its potential as an anti-obesity agent, particularly among obese subjects.

Objective: This review aims to outline the characteristics and findings regarding the use of coconut oil as an anti-obesity agent in both experimental animal and human studies.

Method: The review included original studies on the potential of coconut oil as an anti-obesity agent, published in English or Indonesian between 2011 and 2022. A comprehensive search was performed across databases such as PubMed, ScienceDirect, Google Scholar, SpringerLink, and Portal Garuda. Articles were selected following the PRISMA-ScR flow diagram, with subsequent data extraction, analysis, and synthesis conducted.

Results: Eight articles met the inclusion criteria, comprising four preclinical trials (involving obese animal models) and four human studies that recruited obese people. The preclinical trials used virgin coconut oil (VCO), while the human studies utilized both VCO and coconut oil (CNO). In preclinical trials, VCO administration was associated with reductions in low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglyceride (TG) levels. In clinical trials, consumption of VCO and CNO was linked to increased high-density lipoprotein cholesterol (HDL-C) levels and reductions in LDL-C, TC, TG, and anthropometric measures (body weight, body mass index, waist circumference, waist-to-hip ratio, and body fat mass). However, the effects of VCO and CNO on LDL-C, TC, and TG were inconsistent.

Conclusion: Coconut oil, particularly in the form of VCO, has shown potential as a supplement for managing obesity. However, this scoping review highlights inconsistencies in the effects of coconut oil on lipid profiles (LDL-C, TC, TG), indicating the need for further research to clarify these outcomes.

Keywords: Coconut oil, *Cocos nucifera* L., medium-chain saturated fatty acid, obesity

1. Introduction

The global prevalence of obesity has tripled between 1975 and 2016 (WHO, 2021). A cross-sectional study based on the National Basic Health Survey in 2007 reported that the prevalence of obesity and central obesity among the adult population (aged >18 years) in Indonesia was 23.1% and 28%, respectively (Harbuwono *et al*., 2018). According to data from Basic Health Research and the Statistic Center Agency, the prevalence of obesity in the adult population in Indonesia increased significantly, rising from 19.1% in 2007 to 35.4% in 2018 (Statistic Center Agency, 2021).

The World Health Organization (WHO) defines obesity as excessive and abnormal fat accumulation that negatively impacts health (Panuganti *et al*., 2021). The primary treatment for obesity involves lifestyle management, such as dietary changes, increased physical activity, and behavioral modifications (Hamdy *et al*., 2021). While pharmacological therapy and surgery are additional treatment options, they are associated with higher costs and potential side effects.

Researchers are increasingly interested in exploring natural resources that could provide effective and safe approaches to addressing obesity (Lagerros & Rössner, 2013; Pilitsi *et al*., 2019).

The coconut tree is a natural resource with potential as an anti-obesity agent (da Silva Lima & Block, 2019). It belongs to the kingdom Plantae, order Arecales, family Aracaceae, subfamily Cocoidae, genus Cocos L., and species *Cocos nucifera* L. (Schoch CL, 2020). One of the main products derived from the coconut tree is coconut oil (CNO), which exists in two forms: refined, bleached, and deodorized copra oil (RBDCO or CO) and virgin coconut oil (VCO) (Suryani *et al*., 2020). CNO is rich in saturated fatty acids (SFA), with approximately 60% being medium-chain fatty acids (MCFA), which contain 6 to 12 carbon atoms. The primary MCFA in CNO are lauric acid, myristic acid, and palmitic acid. While there has been considerable research on the potential benefits of CNO, no scoping review has specifically examined its use as an anti-obesity agent. Therefore, this scoping review aims to outline the characteristics and findings regarding the potential of CNO as an anti-obesity agent in both obese animal models and human subjects.

2. Method

The article selection followed the Preferred Reporting Items for Scoping Reviews (PRISMA-ScR) framework (Tricco *et al*., 2018), which includes steps for identification (searching databases using keyword combinations), screening (assessing titles and abstracts to exclude irrelevant articles and remove duplicates), eligibility (excluding articles that did not meet the inclusion criteria), and inclusion (selecting final articles for review).

2.1. Search strategy

A systematic search was conducted using a Boolean search strategy across multiple databases. The keyword combinations used in PubMed, Science Direct, Google Scholar, and SpringerLink were (“coconut oil” OR “Cocos nucifera”) AND (“medium chain triglycerides” OR “medium chain saturated fatty acids”) AND (obesity OR “weight loss” OR overweight). For searching Indonesian articles, we used Google Scholar and Portal Garuda; the keywords used were “minyak kelapa” AND “obesitas”. The search was filtered for articles published between 2011 and 2022.

The article selection process was based on predetermined inclusion criteria, which required articles to be written in English or Indonesian, published between 2011 and 2022, and categorized as original research with experimental study designs. Eligible studies involved either diet-induced obesity animal models or human subjects with obesity (Body Mass Index (BMI) ≥ 25 kg/m^2), regardless of age, gender, symptoms, comorbidities, or complications, and focused on investigating

the potential of coconut oil as an anti-obesity agent. Studies were excluded if they focused on populations not meeting the criteria (e.g., individuals without obesity or animal models not induced with diet-induced obesity), did not use coconut oil as the primary intervention, or were non-experimental studies such as reviews, meta-analyses, commentaries, case reports, or editorials.

2.2. Extraction and data charting

Articles that met the inclusion criteria underwent data extraction by two independent reviewers to minimize bias. The extracted data were compiled into a table in Microsoft Word, with data components including the author, year, country, study design, objective, total sample size, variables, key findings, and conclusions.

3. Result and discussion

3.1. Characteristics and study designs

A total of 656 articles were identified across all databases. After removing duplicates, 623 articles remained. Following a screening of titles and abstracts, 103 articles were considered potentially suitable. The eligibility assessment resulted in 80 full-text articles being reviewed, of which 8 met the inclusion criteria and were included in this review (Figure 1). Among these, 4 were preclinical studies conducted on obese animal models to report the effects of VCO at various doses, while the remaining 4 were clinical trials involving obese people, with 2 assessing the anti-obesity effects of VCO and 2 evaluating the effects of CNO.

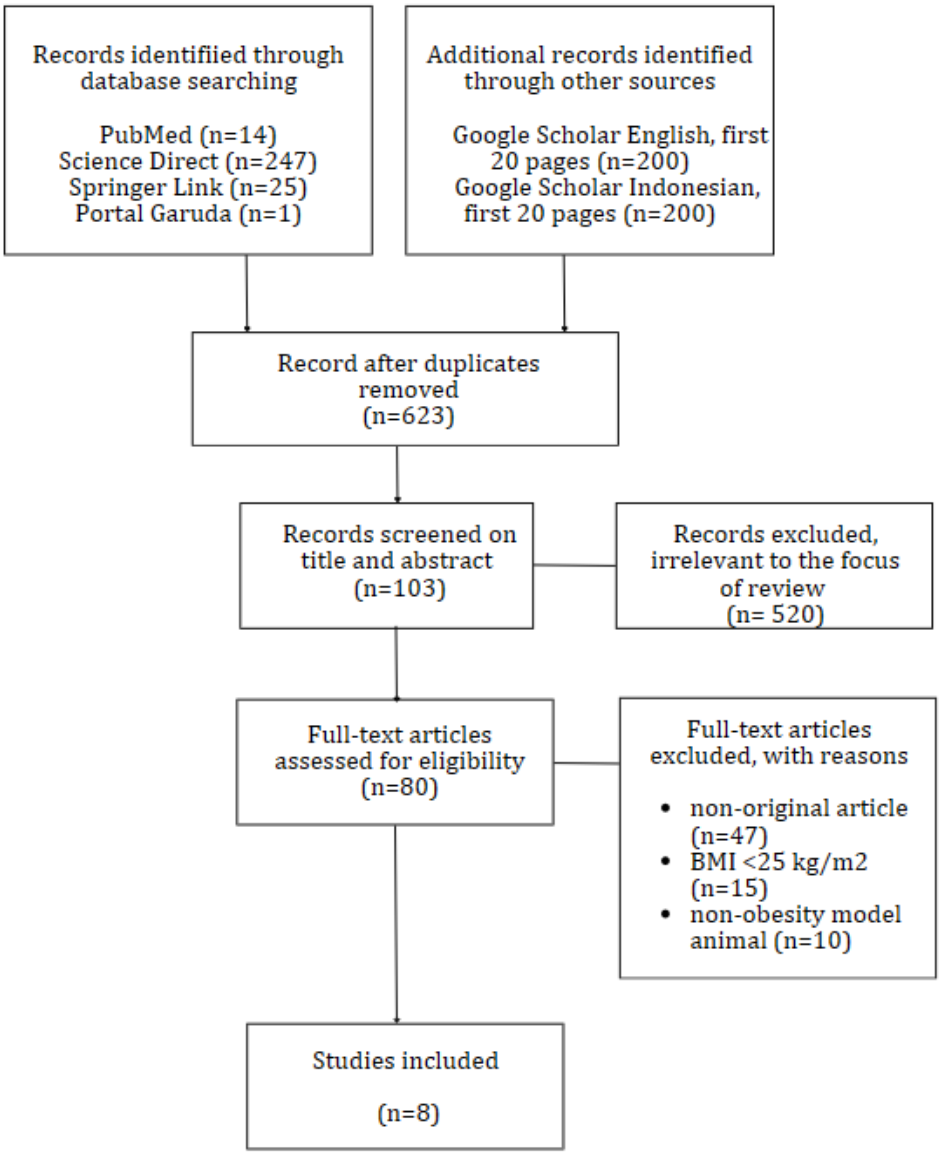

Figure 1. PRISMA-ScR flow diagram (Tricco *et al*., 2018)

Preclinical trials across four studies were conducted in Brazil and Nigeria between 2018 and 2020, involving male Wistar and BALB/c mice as experimental obese models (**Table 1**). The study designs included one multi-group pre- and post-test design (Romão-Carrascoza *et al*., 2019), two multi-group post-test-only design (Adeyemi, Olayaki, Abdussalam, Ige, et al., 2020; Zicker et al., 2018), and one of two-group post-test-only design (Ströher *et al*., 2019). The multi-group pre- and post-test study compared three groups (standard feed vs. high-carbohydrate diet (HCD) vs. HCD with added extra-VCO) (Romão-Carrascoza *et al*., 2019). Two studies employed a multi-group post-test-only design, divided the animal models into six groups (Adeyemi *et al*., 2020) and five groups (Zicker *et al*., 2018). VCO supplementation in Adeyemi's study was provided at doses of

200 mg/kg body weight (BW), 400 mg/kg BW, and 600 mg/kg BW. Zicker's study assessed high-refined carbohydrate (HC) diet with VCO at doses of 1000 mg/kg BW, 3000 mg/kg BW, and 9000 mg/kg BW. The two-group post-test-only study compared a group on an high-fat diet (HFD) with saline to a group on an HFD with VCO (Ströher *et al*., 2019).

Four human studies were conducted from 2018 to 2022 in Indonesia, Iran, Brazil, and Turkey, involving a total of 193 obese people (**Table 2**). The human trials involving VCO interventions used a quasi-experimental design with a pretest-posttest approach (Sinaga *et al*., 2021) and randomized controlled trials (RCTs) (Nikooei *et al*., 2020). The studies involving CNO interventions also employed RCT designs (Koc *et al*., 2022; Oliveira-De-Lira *et al*., 2018). Sinaga's study compared two groups of obese women: aerobic exercise alone vs. combined aerobic exercise with VCO consumption over an 8-week period (Sinaga *et al.*, 2021). The RCT conducted by Nikooei *et al.* used a stratified block randomization method to compare a group receiving a diet with ordinary daily oil and a group receiving VCO (Nikooei *et al*., 2020). Oliveira-de-Lira *et al.* performed a double-blind RCT comparing four groups: a soybean oil (placebo) group, a safflower oil group, a chia seed oil group, and a CNO group. Participants were instructed to reduce their caloric intake by approximately 500 kcal and engage in physical activity, specifically walking for at least 50 minutes, four times per week (Oliveira-De-Lira *et al.*, 2018). In contrast, Koc *et al.* used a two-phase cross-sectional RCT design. During the first phase, one group received CNO along with a hypocaloric diet, while the second group followed only a hypocaloric diet. In the second phase, the interventions were reversed across two groups (Koc *et al*., 2022).

3.2. Animal models

To establish obese mouse models, a HFD was administered for varying durations: 8 weeks (Zicker *et al*., 2018), 10 weeks (Romão-Carrascoza *et al*., 2019), 12 weeks (Ströher *et al*., 2019), and 16 weeks (Adeyemi, *et al*., 2020). Most of the animal models utilized in these studies are male Wistar rats. Female rats experience hormonal fluctuations during their estrous cycle, while male rats generally exhibit a more stable hormonal pattern (Leskanicova *et al*., 2020). The Wistar strain is commonly selected due to its calm and docile nature, as well as its low susceptibility to stress. Wistar rats also have small bodies, so they are easier to accommodate if large numbers are needed. Furthermore, Wistar rats possess a genetically homogeneous profile, thereby reducing confounding factors that could potentially influence the research outcomes (Krubaa & Yogitha, 2024).

3.3. Interventions

The VCO used in the preclinical trials varied in origin and preparation methods. One study used commercially available extra-VCO (Romão-Carrascoza *et al*., 2019). Adeyemi's study used VCO obtained from the Kwara State Ministry of Agriculture and Natural Resources in Nigeria, prepared by grinding coconut endosperm, mixing it with 400 ml of water, and allowing it to separate for one day, after which the supernatant oil was collected (Adeyemi *et al*., 2020). The VCO used by Ströher *et al.* was produced through a pressing and filtration process of coconut fruit (Ströher *et al*., 2019). In Zicker *et al.*'s study, the VCO was sourced from organic samples in Conde, Bahia, Brazil; however, no further details on its preparation were provided (Zicker *et al*., 2018). The differences in the source and preparation of this VCO pose a limitation in comparing the outcomes between studies. VCO and CO differ in several ways. VCO is obtained through the fermentation of fresh, mature coconut flesh, while CO is obtained from the extraction of dried coconut flesh. The purification process for VCO involves simple methods such as washing, settling, filtering, and centrifugation, whereas CO undergoes a refined, bleached, and deodorized (RBD) process. Unlike CO, the production of VCO does not involve heating, which allows VCO to retain a higher content of MCFA and antioxidants. In contrast, the heating process used in CO production breaks down many of the fatty acid carbon bonds and leads to the loss of antioxidants (Mela & Bintang, 2021; Suryani *et al*., 2020).

The duration of the VCO intervention was 4 weeks in three preclinical studies and only one study assessed the intervention for 30 days (Ströher *et al*., 2019). The doses of VCO varied in both quantity and measurement units. Two studies applied a single dose of VCO: 6 mg/100 g BW (Romão-Carrascoza *et al*., 2019) and 2 ml/kg BW (Ströher *et al*., 2019). The other two studies used multiple doses: 200 mg/kg BW, 400 mg/kg BW, and 600 mg/kg BW (Adeyemi *et al*., 2020), as well as 1000 mg/kg BW, 3000 mg/kg BW, and 9000 mg/kg BW (Zicker *et al*., 2018). The dosing units reported were mg/100 g BW, mg/kg BW, and ml/kg BW.

The interventions in human studies included the use of VCO (Nikooei *et al*., 2020; Sinaga *et al*., 2021) and CNO (Koc *et al*., 2022; Oliveira-De-Lira *et al*., 2018). The duration of VCO interventions ranged from 4 weeks (Nikooei *et al*., 2020) to 8 weeks (Sinaga *et al*., 2021), while CNO interventions were administered over 8 weeks (Koc *et al*., 2022; Oliveira-De-Lira *et al*., 2018). In the studies evaluating VCO, the oil was consumed directly at a dosage of 15 mL, 3 times per day (Sinaga *et al*., 2021) or used as a salad dressing or mixed into milk, tea, coffee, or other beverages

at a dosage of 30 mL/day (Nikooei *et al*., 2020). CNO was administered either directly at a dose of 20 mL/day (Koc *et al*., 2022) or in capsule form (Oliveira-De-Lira *et al*., 2018).

3.4. Anti-obesity effects of coconut oil supplementation

In studies involving obese mouse models, the primary parameters assessed were lipid profiles, including low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), total cholesterol (TC), and triglycerides (TG). None of the studies evaluated weight loss in mice following VCO administration. Reported improvements in lipid profiles included reductions in LDL-C (Adeyemi *et al*., 2020), TC (Adeyemi *et al*., 2020; Ströher *et al*., 2019; Zicker *et al*., 2018), and TG (Adeyemi *et al*., 2020; Ströher *et al*., 2019; Zicker *et al*., 2018). However, some studies presented contradictory findings, with increases in LDL-C (Ströher *et al*., 2019) and in TG and VLDL-C (Romão-Carrascoza *et al*., 2019) following VCO administration.

A multi-group study with varied doses of VCO by Adeyemi *et al.* indicated that lower doses were more effective in improving obesity-related parameters in animal models. In contrast, high doses of VCO, which contain greater amounts of saturated fatty acids (SFA), were associated with increased cholesterol accumulation. This accumulation can damage the histological structure of the liver and vascular tissue, create an imbalance between antioxidants and prooxidants, and increase lipid peroxidation (Adeyemi *et al*., 2020; Rosqvist *et al*., 2019; Zicker *et al*., 2018). Apart from CNO, chia seed oil has also been reported to have a positive effect on lipid profiles (HDL-C, LDL-C, TC, and TG). An RCT included in this review demonstrated that chia seed oil resulted in better improvements in lipid profiles compared to CNO (Oliveira-De-Lira *et al*., 2018). Chia seeds are rich in α-linolenic acid (ALA) and linoleic acid (LA), which are known to increase HDL-C and reduce TG levels (Hrnčič *et al.*, 2020; Ullah *et al*., 2015).

In this review, the anti-obesity effects of VCO and CO are attributed to the properties of MCFA, which are absorbed more quickly and converted into energy more efficiently. MCFA can enhance thermogenesis, increase metabolic rate, boost energy expenditure, and promote satiety. VCO contains a higher concentration of MCFA than CO, contributing to its more pronounced anti-obesity effects. This is supported by research from Nevin and Rajamohan, which demonstrated that VCO was more effective than CO in reducing LDL-C, TC, and TG, while also increasing high-density lipoprotein cholesterol (HDL-C). Additionally, the high antioxidant content in VCO may help prevent LDL-C oxidation (Bueno *et al*., 2014; Deen *et al*., 2021; Nevin & Rajamohan, 2004).

The findings in this review indicate a trend of improvements in lipid profiles with the consumption of coconut oil, including increased HDL-C levels and decreased LDL-C, TC, and TG levels. The reviewed studies applied doses ranging from 30-45 mL/day for VCO and 20 mL/day for CNO for 8 weeks. However, there were inconsistent findings regarding the effects on LDL-C, TC, and TG levels. While some studies reported an increase in HDL-C levels in obese patients due to the high saturated fatty acid (SFA) content in VCO or CO, others found that the high SFA content also led to increased LDL-C and TC levels. These inconsistencies highlight the need for further research to clarify the effects of VCO and CO consumption, particularly concerning cardiovascular disease risk (Chinwong *et al*., 2017; Briggs *et al.*, 2017).

The primary parameter for assessing obesity was anthropometry, including measurements of BW, body height, BMI, waist circumference (WC), and waist-to-hip ratio (WHR). One study found that CNO consumption significantly reduced BW, BMI, WC, and WHR in obese patients (Oliveira-De-Lira *et al*., 2018). Participants consuming CNO showed the most substantial reductions in BW, BMI, WC, WHR, and the conicity index (CI) compared to those consuming safflower oil, chia seed oil, or soybean oil. This study also reported that CO consumption resulted in a weight loss of ≥5% in 94.4% of participants and a weight loss of ≥10% in 22.2% of participants, with statistically significant results ($p < 0.05$). In contrast, the studies by Koc *et al*. (2022) and Nikooei *et al*. (2020) found that VCO or CNO administration did not affect anthropometric parameters, and the study by Sinaga *et al*. (2021) did not report on these parameters. The potential effect of coconut oil on improving anthropometric parameters is attributed to the MCFA it contains, which are more rapidly absorbed and metabolized in the liver into energy, rather than being stored as fat in adipose tissue (Deen *et al*., 2021). Additionally, MCFA can enhance thermogenesis, the process of heat production from fat, leading to a reduction in body fat mass. Thermogenesis primarily occurs in brown adipose tissue, where its main regulator is uncoupling protein 1 (UCP1). Some studies have reported that MCFA can increase UCP1 expression, thereby enhancing thermogenesis (Dayrit, 2014; Ikeda & Yamada, 2020).

Lifestyle modification is the primary intervention for managing obesity. Three articles included in this scoping review reported that, in addition to interventions using VCO or CNO, subjects were also encouraged to adopt lifestyle modifications, including dietary adjustments and increased physical activity (Koc *et al*., 2022; Nikooei *et al*., 2020; Sinaga *et al*., 2021). Dietary regulation, such as calorie restriction or intermittent fasting, plays a crucial role in managing obesity. Alongside dietary changes, physical activity is another essential component of lifestyle modification. Physical

activity stimulates the release of epinephrine and norepinephrine, which promotes lipolysis, the breakdown of fat stores (Polak *et al*., 2008; Thompson *et al*., 2012).

The studies included in this scoping review present findings from both experimental animal and human research on the effects of coconut oil, particularly VCO, on obesity-related parameters. The strength of this scoping review lies in its focus on subjects with obesity, providing targeted insights into the potential anti-obesity effects of CNO and VCO. However, this review has some limitations. It only included articles published in English or Bahasa Indonesia, which may have resulted in the exclusion of relevant studies from other countries published in different languages. This restriction could potentially lead to missing valuable data and perspectives from a broader range of research.

4. Conclusion

This review highlights the potential of coconut oil, particularly in the form of VCO, as a supplement for managing obesity. VCO supplementation shows beneficial effects on lipid profiles and obesity-related parameters by leveraging its MCFA content. However, the findings also reveal inconsistencies, particularly regarding the impact of coconut oil on lipid profiles, such as LDL-C, TC, and TG, which underscore the need for further research. To optimize weight management and overall health outcomes, incorporating lifestyle modifications such as a balanced diet and regular physical activity alongside VCO supplementation is strongly recommended.

Table 1. Data extraction in preclinical trials

No.	Author, year	Country	Study design	Animal obese model	Intervention	Parameters	Key findings
1.	Romão-Carrascoza *et al*. (2019)	Brazil	Multi group pre and posttest design	Male Wistar rats (n = 35), obesity model with HCD	1. Saline 2. HCD + saline 3. HCD + extra-VCO 6 mg/100 kg BW, at 10.00 AM and 5.00 PM for 4 weeks	BW gain, adiposity index, adipocyte diameter, liver lipid contents, plasma lipid profile, glycemia, insulinemia, formation of AGEs	VCO supplementation decreases visceral adipocyte size, AGE formation, NAFLD, glycemia index, insulinemic index, liver lipid contents, and fructosamine level. VCO supplementation improves insulin levels, insulin sensitivity, VLDL-C, and TG.
2.	Adeyemi *et al*. (2020)	Nigeria	Multi group posttest only design	Male Wistar rats (n = 60), obesity model with HFD	1. Saline 2. HFD + saline 3. HFD recovery 4. HFD + VCO 200 mg/kg BW 5. HFD + VCO 400 mg/kg BW 6. HFD + VCO 600 mg/kg BW for 4 weeks	Hepatic histoarchitecture, blood glucose, insulin, insulin resistance, lipid profile (TC, TG, LDL-C, HDL-C), atherogenic index, Lee index, albumin, total protein, total bilirubin, liver enzyme (AST, ALT, ALP, GGT)	VCO, preferably at a low dose (200 mg/kg BW) improves hepatic structural alteration and biochemical deviations. VCO increases SOD and decreases LDL, TC, TG, LDL-C, atherogenic index, AST, and ALT. However, the MDA, IL-6, CRP level decreased.
3.	Ströher *et al*. (2019)	Brazil	2 group posttest only design	Male Wistar rats (n = 12), obesity model with HFD	1. HFD 2. HFD + VCO 2 ml/kg BW for 30 days	Biochemical parameters (TC, HDL-C, LDL-C, TG, glucose, ALT, AST), liver lipid contents, hepatic TC, hepatic TG, oxidative stress, histology (liver, adipose tissue, aorta)	HFD + VCO supplementation increases diet intake, LDL-C , TG, adipose inflammatory gene expression, AST and ALT levels, weight gain, hepatic fat accumulation, lipid peroxidation, and adipocyte hypertrophy. VCO reduces TC and glucose levels and increases oxidative stress parameters (SOD, CAT. GPx)
4.	Zicker *et al*. (2018)	Brazil	Multi group posttest only design	Male BALB/c mice (n = 40), obesity model with HC	1. Chow diet 2. HC 3. HC + VCO 1000 mg/kg BW 4. HC + VCO 3000 mg/kg BW 5. HC + VCO 9000 mg/kg BW	Adiposity levels, adipocytes size, VO_2, EE, RER, hematology (leukocytes, peripheral blood smear), epididymal adipose tissue, , blood glucose, TC, TG, ALT, AST, adipokine (chemerin, adiponectin, resistin, leptin), cytokine (TNF-α, IL-1β, IL-4, IL-6, IL-10, IL-13, TGF-β), hepatic and adipose tissue histology, total hepatic lipid	VCO supplementation improves metabolism abnormality (glucose homeostasis, serum lipid profile, hepatic steatosis, hepatic TC, TG, ALT), inflammatory response (leukocytes, mononuclear cell, TNF-α, IL-6, macrophage), and adiposity. VCO

No.	Author, year	Country	Study design	Animal obese model	Intervention	Parameters	Key findings
					4 weeks		supplementation has no effect on VO_2, EE, and RER.

high carbohydrate diet (HCD), virgin coconut oil (VCO), advanced glycated end-products (AGEs), non-alcoholic fatty liver disease (NAFLD), very low density lipoprotein cholesterol (VLDL-C), triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), malondialdehyde (MDA), low density lipoprotein (LDL), interleukin-6 (IL-6), C-reactive protein (CRP), high fat diet (HFD), high-refined carbohydrate (HC), oxygen consumption (VO_2), energy expenditure (EE), respiratory exchange ratio (RER), tumor necrosis factor alpha (TNF-α), interlukin-1 beta (IL-1β), interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-13 (IL-13), transforming growth factor beta (TGF-β),

Table 2. Data extraction in clinical trials

No.	Author, year	Country	Study design	Subject	Intervention and duration	Parameter	Key findings
1.	Sinaga *et al*. (2021)	Indonesia	Quasi-experimental design with pretest-posttest design	Obese women (n = 20), aged 43.55 ± 2.21, BMI 31.69 ± 1.63	1. VCO 15 ml 3x/day 8 weeks + aerobics 2. Aerobic	Triglyceride, cholesterol	Aerobic exercise and VCO supplementation can reduce TG dan TC levels.
2.	Nikooei *et al*. (2020)	Iran	Randomized controlled clinical trial	Male and female (n = 48), aged 20-50 years, metabolic syndrome	1. VCO 30 ml per day, consume it for mild cooking and salad dressing or mix with milk, coffee, tea, etc. (avoid overheating), 4 weeks 2. Placebo/control (routinely consumed oil)	Anthropometric (weight, height, BMI, WC), body composition (BFM, VFL, SMM), blood glucose, lipid profile (TC, TG, LDL-C, HDL-C, VLDL), ADMA	VCO supplementation increases HDL-C, LDL-C, ADMA, and TC levels and reduces TG and VLDL levels. VCO consumption has no effect on blood pressure, body composition, and anthropometry.
3,	Oliveira-De-Lira *et al*. (2018)	Brazil	Randomized, double-blind, placebo-controlled clinical trial	Women (n = 75), aged 20-40 years, BMI ≥30 kg/m^2 and ≤39,9 kg/m^2	1. Control = soybean oil capsule 2. Safflower oil capsule 3. Chia oil capsule 4. CNO capsule, 8 weeks	Weight, height, WC, BMI, WHR, %BF, %LM, %water, CI, TC, LDL-C, HDL-C, TG, HbA1c, TG/HDL ratio, MEG	CNO reduces abdominal fat, weight, BMI, WC, WHR, CI, %BF and improves glycemic parameters (HbA1c, MEG) and lipid profiles (TC, TG, LDL-C, HDL-C).
4.	Koc *et al*. (2022)	Turki	Randomized controlled trial, two-phase cross-sectional design	Adult (n = 44), aged19-30 years, BMI 25-29,5 kg/m^2	1. Hypocaloric diet + CNO 20 ml/day, 4 weeks in phase 1 and 4 weeks in phase 2	Weight, WC, BMI, detailed body analysis, WHC, TC, HDL-C, TG, LDL-C, insulin, irisin	CNO has no effect on anthropometric measurement (weight, BMI,

No.	Author, year	Country	Study design	Subject	Intervention and duration	Parameter	Key findings
					2. Control = hypocaloric diet		WHR). CO reduces level irisin, insulin, TC, and LDL-C.

virgin coconut oil (VCO),coconut oil (CNO) body mass index (BMI), total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein (VLDL), asymmetric dimethylarginine (ADMA), waist-to-height ratio (WHR), percentage of body fat (%BF), lean mass (%LM), conicity index (CI), hemoglobin A1c (HbA1c), mean estimated glycemia (MEG)

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The role of pharmacists at the implementation of transformational leadership in the hospital central sterilization

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Abstract

Background: Pharmacists in hospitals have a role that is not only limited to the pharmacy installation and management but also contributes to the *Central Sterile Supply Department* (CSSD) installation. The issue of sterilization and cleanliness of instruments and linen in hospitals is the responsibility of the CSSD to support the sustainability of quality services in hospitals. Although related to sterility, most of the staff of the installation CSSD in Indonesia are high school graduates with minimal health knowledge and are often transferred from other installations due to problems. Some hospitals combine CSSD with laundry; in contrast to the pharmacy installation, which actively involves pharmacists and pharmaceutical technical personnel, compliance with regulations and provisions is more highly respected compared to personnel in the CSSD installation.

Objective: This study aims to analyze the application of transformational leadership style in CSSD installation in hospitals, while highlighting the lack of leadership studies in pharmacy and the effectiveness of leadership in managing installation performance in hospitals with non-health personnel.

Methods: The methods used include direct observation, interviews, and questionnaires with samples consisting of installation leaders and staff CSSD in hospitals. We explore the factors influencing and challenges faced in implementing transformational leadership in a hospital CSSD environment.

Results: The questionnaire results showed that the transformational leadership scores of the leaders were high, and most employees were satisfied with the implementation of transformational leadership. Some management concepts have been applied to improve performance, but many have not been able to provide change due to a lack of response from employees.

Conclusion: A leader in CSSD installation must be flexible in adopting a leadership style that suits the situation and conditions; the application of leadership style must consider internal factors such as human resources quality and organizational structure as well as external factors that are hospital management and budget support.

Keywords: Leadership style, transformational leadership, hospital central sterilization and laundry, health service management

1. Introduction

The role of pharmacists in hospitals can be in the management section, pharmacy installation, Central Sterile Supply Department (CSSD) installation and other installations related to the pharmaceutical profession. Pharmacists in hospitals can play a structural or functional role so that pharmacists can be placed outside the pharmacy installation. In addition to the Minister of Health Regulation Number 72 of year 2016 of Minister of Health (2016) concerning Hospital Service Standards, the functional position of pharmacists in hospitals is regulated in Minister of State Apparatus Utilization and Bureaucratic Reform Regulation Number 13 of 2021, elements of the pharmacist functional position task activities that can be assessed for credit points include preparation of pharmacy practice plans; management of pharmaceutical preparations, medical

devices, and BMHP; clinical pharmacy services; central sterilization; application of pharmacoeconomic studies and clinical trials; and special pharmacy services.

As for other health workers, there is no description for their functional positions in the CSSD. Pharmacists in CSSD installations usually act as leaders, furthermore in some hospitals, due to interrelated work activities, CSSD installations are often combined into one installation with hospital laundry. According to Hospital Sterilization Center Installation Guidelines of Ministry of Health of the Republic of Indonesia (2009) and PPSSI (2023), the CSSD leadership standard is filled by health workers while the laundry is filled by non-health workers Holdford (2018) stated that most pharmacy textbooks do not address the topic of leadership or only mention it briefly.

Six basic leadership styles that can be applied to change work situations have been identified: coercive, transformational, affiliative, democratic, pacesetter, and coach (Goleman, 2000). Six styles of leadership proposed by Goleman, Goleman's description of leadership types according to Cwalina & Drzewiecka (2015) is described in **Table 1** as follows:

Table 1. Six types of leadership styles

Leadership style	Main characteristics	The style in phrase
Coercive	Demands immediate compliance, has overall negative impact on climate in organization, works good in time of crisis or in case of problems with workers	"Do, what I tell you"
Authoritative	Mobilizes people toward vision, works especially well, if new vision or new direction is required	"Come with me"
Affiliative	Concentrates on harmony and builds emotional bonds, works well, when people face difficult circumstances	"People come first"
Democratic	Forges consensus through participation and works successfully, if there is a need to build buy-in or in consensus, or to get input from valuable team members	"What do you think"
Pacesetting	Style sets high standards for performance, works effectively with motivated and competent people, knowing, how to get quick results from them	"Do it, as I do, now"
Coaching	Develop people for future, works especially well if there is a need to help others improving performance or set- ting long term strengths	"Try this"

Source: Cwalina & Drzewiecka, 2015

Bass (1985) divides leadership into two types, namely transformational leadership and transactional leadership. Bass argues that transformational leadership is more effective than transactional leadership in terms of the greater contribution of transformational leaders in motivating group members compared to transactional leaders. Compared to subordinates, transformational leaders provide greater satisfaction than transactional leaders because subordinates not only need to be paid after completing the work, but they also need attention, intellectual stimulation, and advice obtained from their leaders (Bass and Avolio 1993).

According to Sethibe and Steyn (2015), leadership style has been recognized as one of the most important factors influencing the relationship between innovation and organizational

performance, because leaders have the authority to set specific goals and encourage innovation. Eliyana *et al.* (2020) stated that to realize innovative work behavior in employees, assistance from a leader is certainly needed, especially a leader with a transformational leadership style. Transformational leadership is believed to be one of the main driving factors for employees to be able to innovate. This is because in general leaders with this leadership style have an open mind and are always oriented towards future planning, so that employees are definitely encouraged to be more innovative in their work.

Sterility and cleanliness of medical devices and linen in hospitals are the main things to support the sustainability of hospital quality, on the other hand, most of the human resources employed in this field are employees with minimal knowledge about health and are often considered a waste of other installations in the hospital. In contrast to the pharmaceutical installation which plays a large role as a pharmacist, order and compliance with the rules are more highly respected than in the CSSD installation. This is the background of this study, in addition to implying it also confirms that there has been no research on leadership in pharmaceutical research and the effectiveness of leaders in managing the performance of installations consisting of other health workers. This basis then triggered the need for research on the transformational leadership of the head of the CSSD installation and laundry. Therefore, this study aims to analyze the application of transformational leadership in the CSSD installation and hospital laundry and its impact on the performance and job satisfaction of officers.

2. Method

2.1. Sample preparation

This study was conducted on the leaders and employees of the CSSD installation which is one with the laundry (1 Administrative Staff and 19 Executive Staff) in one of the government hospitals with type B education in East Java, Indonesia, which has capable employees and sophisticated sterilization equipment, with a number of patient beds of 300 beds and an operating room with 20-25 patients every day. The inclusion criteria for this study were the head of CSSD and laundry installations and their staff who filled out the questionnaire form, while the exclusion criteria were employees who were on leave or sick.

2.2. Method

The type of research used is qualitative descriptive. Data collection techniques using interviews, observations and questionnaire. Observations will be recorded and analyzed to see

patterns of leadership practices. Interviews with leaders and staff will be recorded and translated to gain in-depth insights into the experiences and views of respondents. MLQ (Multifactor Leadership Questionnaire) in accordance with Bass's transformational questionnaire (1985) is used to measure the implementation of transformational leadership. The questionnaire consists of 36 questions with answers in the form of a Likert scale.

The questionnaire used is The LMX (Leader-Member Exchange) questionnaire is used to measure the relationship between leaders and subordinates. There have been many standard instruments used in measuring LMX, one of which is the LMX-MDM for leaders and followers developed by Liden & Maslyn (1998). This instrument applies the dyadic concept by analyzing not only the leader's perspective but also the follower's perspective. To determine the quality of LMX in CSSD and laundry installation, the leader respondents (head of installation) assessed the quality of their relationship with members (government employees and contract employees) and vice versa according to the LMX questionnaire, namely 12 specific questions for leaders and 12 specific questions for members with answers in the form of a Likert scale.

2.3. Data analysis

The results of the MLQ questionnaire will be processed by adding up the scores of transformational, transactional and leize faire leadership values according to the Bass transformational questionnaire (1985). While the LMX diagram is used to determine the interpretation of the relationship between LMX from the perspective of the leader and member, namely by linear regression between leader (X) and member (Y) will be processed using SPSS software.

3. Results and discussion

3.1. Results

The CSSD installation and hospital laundry installation consist of one head of the installation, coordinator, head of sub-installation and Government Employees and Contract Employees. From the interview and questionnaire that was distributed, the following data (**Table 2**) was obtained:

Table 2. Respondent profile of CSSD and laundry installation

Respondent	Education / graduation year	Gender	Unit
Respondent 1	S1 Environmental Engineering /2017	F	Administration
Respondent 2	S1 Management /1996	M	Distribution
Respondent 3	S1 Industrial Engineering / 2004	M	Distribution

Respondent	Education / graduation year	Gender	Unit
Respondent 4	S1 Chemical Engineering /2004	M	Warehouse
Respondent 5	High School / 1991	M	CSSD
Respondent 6	High School / 2011	M	CSSD
Respondent 7	High School / 2017	M	CSSD
Respondent 8	High School / 2015	M	Distribution
Respondent 9	High School / 1994	M	Laundry
Respondent 10	High School / 2015	M	Laundry
Respondent 11	High School / 2004	M	Laundry
Respondent 12	High School Package C/ 2015	M	Laundry
Respondent 13	High School Package C/ 2010	M	CSSD
Respondent 14	Vocational High School /2012	M	CSSD
Respondent 15	Vocational School of Electricity/ 2001	M	CSSD
Respondent 16	Vocational High School/ 2015	F	Distribution
Respondent 17	Vocational High School/ 2017	M	Laundry

From the 17 respondents, employees from the CSSD and Laundry installation consisted of 4 S1 graduates, 7 high school graduates, 2 high school equivalent package C graduates and 4 vocational school graduates.

Table 3. Interview results on transformational leadership aspects at CSSD and laundry installation

No	Aspect	Program	Results	Challenge
1	Building trust (idealized influence - attributes)	Routine satisfaction questionnaire Conduct regular meetings	Meet and eat together, a get-together event every end of year	Some employees have problems with money
2	Acting with integrity (ideal influence - behavior)	Create an integrated consumable product procurement and selection system.	Financial management and profit sharing can be better organized.	The linen procurement budget is still held by another department so it cannot be centralized
3	Motivating others (inspirational motivation)	Conducting employee rotation and training	Internship program at CSSD for all laundry staff and vice versa.	Transfer officers from other installation who are not qualified find it difficult to develop
4	Encouraging innovative thinking (intellectual stimulation)	Digitalization of administration preparation of the 2023 linen centralization plan	Leveraging Google Spreadsheets and SID	Lack of financial support and management systems
5	Individual training & development (individual consideration)	Education and training of CSSD officers and laundry installation	Inhouse training every year	Time is limited due to work

The data above is the result of interviews with the heads of CSSD and laundry installation about what they did when they were in their positions, with reference to aspects of transformational leadership.

Table 4. Results of MLQ questionnaire analysis

Score	Max	Results	Type	Leadership	Information
16.42	20	High	Idealized influence (associated)	Transformational	Ideal influence (attribution and behavior): the leader holds the trust of subordinates, maintains confidence and respect, shows dedication, inspires hopes and dreams, and acts as a role model for subordinates.
18.42	20	High	Idealized influence (behavior)		
18.83	20	High	Inspirational motivation (IM)	Transformational	Inspirational motivation (IM): measures the extent to which the leader provides a demanding vision and goals, uses appropriate symbols and images to help others focus on their work, and tries to make others feel their work is important.
16.83	20	High	Intellectual stimulation (IS)	Transformational	Intellectual stimulation (IS); indicates the extent to which the leader encourages subordinates to be creative in looking at old problems in new ways, creates an environment that is tolerant of extreme positions, and encourages people to question their own and management's values and beliefs.
15.08	20	High	Individualized consideration (IC)	Transformational	Individual consideration (IC): the extent to which the leader shows interest in the well-being of others, assigns tasks individually, and pays attention to those who are less involved in the group.
14.17	20	Moderate	Contingent reward (CR)	Transactional	Conditional rewards (CR): indicates the extent to which the leader tells others what to do to be rewarded, emphasizes what is expected of them, and recognizes their achievements.
16.83	20	High	Management-by-exception: active	Transactional	Monitoring for deviations and errors management by exception (active): assesses whether the Leader informs others of job requirements, is satisfied with standard performance, and believes in "if it isn't broke, don't fix it."
11.83	20	Moderate	Fights Fires (exception: passive)	Passive	Measures how often leaders wait for problems to arise before taking corrective action.
9.83	20	Moderate	Laissez faire (LF)	Passive	Laissez-faire (LF): measures whether the leader requires little help from others, is content to let things run their course, and lets others do their own thing.
12.92	15	High	More effort	Extra Effort	Just ask your subordinates
16.25	20	High	Effectiveness	Formerly effectiveness	Just ask your subordinates
7.42	10	High	Satisfaction	Satisfaction with the leadership	Just ask your subordinates

The hospital CSSD installation is part of low-level management, namely low-level management is the level of implementing management. The results of MLQ questionnaire in **Table 4** that obtained high transformational leadership values, moderate transactional leadership and also moderate laissez faire leadership.

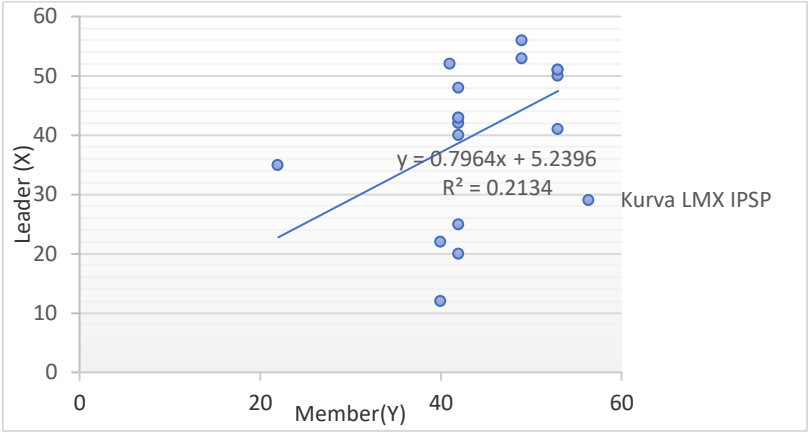

Figure 1. LMX regression graph of CSSD and laundry installation

The data above is the result of linear regression from the MLX questionnaire between leaders and members of the CSSD and laundry installation. The results of the two assessments are mapped in **Figure 2**. The regression results are y = 0.7964x + 5.2396, R^2 = 0.2134.

3.2. Discussion

Table 5. The results of the comparative interview of conditions before and after the implementation of transformational leadership with the parameters of the "New Model of Ishikawa Diagram for Quality Assessment"

Management elements	Definition	Before	After	Challenge
Measurement	Measurement and calculation issues, including data input errors and performance system flaws	Many complaints about missing linen	Linen is not lost but often damaged due to prolonged use but unreported. Now, reporting records are maintained and the items are returned to the supply department	Limited budget
Material	Problems related to raw materials, both in terms of quality, quantity and storage.	Raw materials planning is not well organized	Start organizing warehouse stock and control incoming and outgoing stock according to the computer system.	Unnoticed by management
Machine	Related to the equipment and machinery in the production process.	Underutilized machinery	Running all machines to work optimally and performing routine maintenance.	Maintenance by the IPS department, but financial resources remain constrained
Environment	External factors that are difficult to control, such as weather, natural disasters, or pandemic situations.	Production space cramped and uncomfortable.	Trying to optimize existing conditions	Limited budget
Manpower	Factors related to employees, such as skills, workforce size, or performance issues.	Under skill human resources	Rotate and see employee potential so that it can be optimal.	No employees position rotation

Management elements	Definition	Before	After	Challenge
Method	Relating to company operations, including production, marketing and distribution processes.	Different locations of production and distribution, require daily linen transfers from 1st to 4th floor.	The relocation of linen distribution to the 1st floor resulted in the elimination of transporters, making employees more efficient.	Organizational restructuring is hindered by gaps in staff skills, and some human resources are difficult to develop.

Employees in the CSSD installation and laundry have met the requirements of the 2009 hospital sterilization guidelines but there are still some problems, especially the quality of the employees. Some employees who are transfers from other units have a lower work ethic compared to employees who have been placed in the CSSD and laundry installation from the beginning. This results in bad behavior being transmitted to other employees. During an interview with the head of the CSSD installation, it was found that several management concepts had been implemented but many had not been able to provide change due to the lack of response from employees. Ministry of Health of the Republic of Indonesia (2004) in linen management guidelines in hospitals, the source of infection is the behavior of hospital staff, namely having little or no understanding of how disease is transmitted, paying little or no attention to cleanliness, paying little or no attention to aseptic and antiseptic techniques, suffering from a disease, not washing hands before or after doing work.

In the results of the observation of the comparison of conditions before and after the implementation of transformational leadership with parameters in **Table 5** "New Model of Ishikawa Diagram for Quality Assessment" by Liliana (2016), several obstacles were found, such as human resource quality factors, hospital management support, budget and organizational structure that affect the achievement of the head of the CSSD installation and laundry targets.

Table 6. Microbiological quality of clean linen and instruments sterile at CSSD and laundry installation

No	Type of linen/instrument	Laboratory test results (CFU/100cm^2)	Germ count standard (CFU/100cm^2)	Category
1	Inpatient blanket	12	20	qualify
2	Inpatient room linen	2	20	qualify
3	Inpatient room pillowcase	<1	20	qualify
4	Isolation room blanket	<1	20	qualify
5	Isolation room bed sheet	2	20	qualify
6	Isolation room pillowcase	2	20	qualify
7	Big duk	<1	20	qualify
8	Hole duk	4	20	qualify
9	Gown surgery	<1	20	qualify
10	Endoscopy room scope	8	20	qualify
11	Hollow fiber hemodialysis	<1	20	qualify
12	Dental and oral pliers	<1	20	qualify

According to India National Health Systems Resource Centre (2022), despite all measures and advancements in technology, hospital acquired infections remain a challenge in healthcare scenario today. Sharma et al (2022) explained that the microbial contamination of linen is common, and there is an urgent need to develop consensus on the microbial examination frequency and acceptable range of organisms on hospital linen worldwide. According to Minister of Health Regulation Number 7 of year 2019 of Minister of Health (2019), the microbiological value in linen and instruments determines the quality of the washing and sterilization results. Microbiological measurements of clean linen in CSSD and laundry installation are not carried out periodically due to the lack of budget from the hospital. After implementing transformational leadership, control was carried out in each production process using washing indicators and laboratory tests. In laboratory tests on clean linen, the highest number of germs was found on inpatient blankets, namely 12 CFU/100cm^2. We collect this data by taking swab samples from instruments and linen, then we test them in a laboratory outside the hospital by the sanitation department. The measurement results still meet the standard for the number of germs set by Minister of Health Regulation Number 7 of 2019 No. 7 of 2019, namely the standard for the number of germs on clean linen and medical personnel uniforms after leaving the washing process does not exceed 20 CFU/100cm^2.

The exchange of leaders and members that occurs at this level is formed from the relationship between the head of the work installation (as a leader) and his direct subordinates (as members). The relationship will form an in-group. A comfortable working atmosphere in an in-group will be able to improve the performance of members (Minsky, 2002). Interpretation of **Figure 2** shows that in the CSSD installation and laundry installation an in-group has been formed. According to Putri & Rochmah (2012) that is meant by in-group is a group of members who always work together with the leader in various matters so that the quality of LMX with the leader is good. While members who are in the out-group indicate that members rarely work together with the leader.

Although the regression coefficient value is small, from the LMX graph with an area limited by the line y=x+0.5SD, the number of regression points is greater in the positive balance position. In this condition, it is shown that the assessment between the leader and the members is equally high. The relationship is considered to have good quality (Maslyn & Uhl-Bien, 2001). MLX graphic results on CSSD and laundry installation describe most employees are included in the in-group. From most respondents, it resulted in a balanced positive where subordinates are close to the installation leader. Closeness to the installation leader can potentially lead to positive performance for the implementation of activities in the CSSD and laundry installation. In addition, most employees feel satisfied with the implementation of transformational leadership.

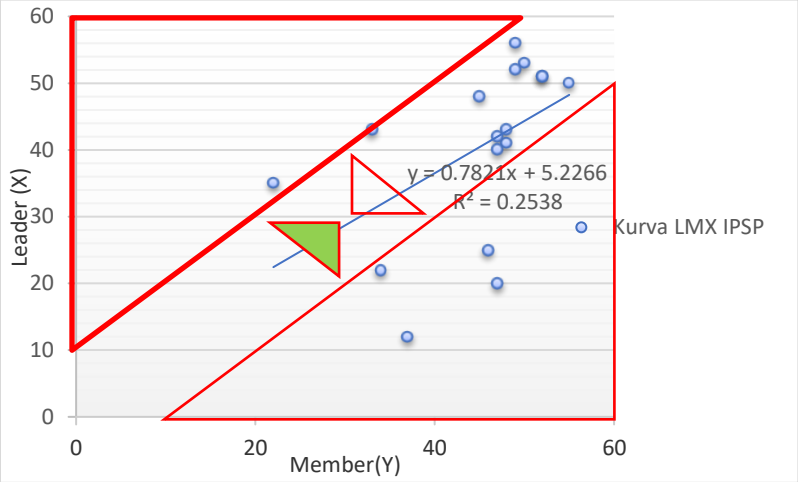

Figure 2. LMX CSSD and laundry installation

Several members fall into the unbalanced formal overinvestment area, this area is limited by the line y=x-0.5SD. This condition occurs when the leader gives a higher value than the member while the member gives a very low value for the leader. Dockery & Steiner (1990) and Day & Crain (1992) stated that leaders who give higher values than members expect a better working relationship. There are several officers who rated less well, when tried to be confirmed gave the answer that the leader should care more about his subordinates. Meanwhile, when cross-checked with the leaders of the CSSD installation and laundry installation, some of these employees if there is no reward are less responsive if given additional work by their superiors (transactional) except for daily routine work. Gones (2016) stated that in health facilities where workers are skilled and accustomed to working in a certain way, staff may tend to be more resistant to change. Second, the emphasis on regulation can maintain staff ownership and commitment to work. Some literature suggests transformational leadership is when a leader inspires people to follow them through vision, passion, and enthusiasm. Other studies suggest a combination of transactional and transformational leadership may be applied in different contexts. This research can be continued with organizational culture assessment indicator (OCAI), according to Masood *et al.* (2006), the OCAI questionnaire was used to obtain an insight into the organizational culture based on the OCAI typology, namely adhocracy, clan, hierarchical, and market.

4. Conclusion

From the results of the questionnaire, the transformational leadership value of the leader is classified as high indicating that most employees are satisfied with its implementation. The leadership style takes into account external influencing factors, requiring leaders to be flexible in

adapting their approach to suit specific situations and conditions. Key factors influencing leadership implementation in the CSSD include human resource quality, internal structure, hospital management support, and budget constraints. This study employs a qualitative method with a survey approach in the CSSD installation of a hospital, providing findings that could be expanded to a broader population.

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Analysis of active compounds in kawista leaf (*Limonia acidissima* L.) fraction and its antioxidant activity using DPPH method

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Abstract

Background: Kawista (*Limonia acidissima* L.) is a plant that has been studied for its potential as a natural antioxidant. Research regarding kawista leaves has not been carried out to a more specific stage, so further fraction separation is carried out to determine compounds that have potential as antioxidant agents.

Objective: This study aimed to determine the antioxidant activity of kawista leaf fractions using the DPPH method and to determine the active compounds in the highest antioxidant agents of kawista leaf fracton.

Method: Kawista leaves were extracted by re-maceration using methanol, which was then purified by the liquid-liquid extraction method with the solvents n-hexane, ethyl acetate, and water. Phytochemical studies were carried out on the fractions using TLC on flavonoids, steroids, tannins, saponins, and alkaloids. The fraction was separated by the vacuum liquid chromatography method with 11 eluents, which were then tested for antioxidants and characterization of the most active compounds in the kawista leaf fraction. Then the highest results as antioxidant agents from the fraction were characterized for their active compounds using a spectrophotometer and FTIR.

Results: The results of the study showed that the most active purified extract in antioxidant activity was ethyl acetate, which indicates strong antioxidant activity. The combined fraction 2 (F2) indicates flavonoid compounds that played an active role as very strong antioxidants with an AAI value of 3.3808, which is not significantly different from the vitamin C as standard. Flavonoid compounds that have been characterized using a spectrophotometer interpret the wavelength shift data that possible F2 belongs to the flavanon group.

Conclusion: Active compounds from kawista leaf fraction (F2) have the highest antioxidant agents, with the possibility that flavonoid compound from F2 belong to the flavanon group.

Keywords: Antioxidant, DPPH, fraction kawista leaf, *Limonia acidissima*

1. Introduction

Free radicals or body oxidants are very dangerous. In fact, free radicals are simply compounds or molecules with one or two unpaired electrons in the outermost level of their atomic structure. These compounds are highly reactive because they have many unpaired electrons. Cell damage or uncontrolled cell development can arise from the binding or attack of molecular electrons by free radicals on nearby molecules such as lipids, proteins, or DNA (the carrier) (Sayuti & Yenrina, 2015).

There are two types of antioxidants: those derived from natural sources and those obtained through chemical synthesis. However, concerns about the side effects of synthetic antioxidants have prompted a shift towards natural antioxidants. Kawista (*Limonia acidissima* L.) is a plant that has been studied for its potential as a natural antioxidant. Kawista is known as one of the most important medicinal plants in India, with one part of the plant, namely the leaves, being used as an antibacterial (Panda *et al*., 2013), diuretic (Parial *et al*., 2009), and for hypoglycemia (Joshi *et al*., 2009).

The Kawista fruit's leaves, stems, fruit skin, and flesh all contain substances that have antioxidant activity, including tannins, steroids, alkaloids, flavonoids, and glycosides. This information is based on the study "Phytochemical screening, antibacterial and antioxidant activity of *L. acidissima* L." (Patil *et al*., 2012). The DPPH technique is used to measure antioxidant activity. Research related to the activities of the kawista plant (*L. acidissima* L.) that has been conducted includes antidiabetic activity, antitumor activity (Eluru *et al*., 2015), antibacterial activity (Anebaracy et al., 2015), and antioxidant activity (Ilango & Chitra, 2010). Research on the antioxidant activity of the kawista plant has been conducted year after year. Antioxidants in kawista fruit were studied by Ilango and Chitra (2010) using methanol extracts of kawista fruit with FRAP and DPPH methods. Meanwhile, Mandade *et al*. (2013) used the DPPH and ABTS methods.

According to Patil *et al*. (2012), antioxidant activity was also found in the leaves, roots, and skin of the kawista fruit. According to Tjahjandarie *et al*. (2017), the compound with antioxidant activity is coumarin found in kawista roots. Maceration with 96% ethanol was used to extract the leaves and peels of the kawista fruit (Rahmi & Rahmadewi, 2020), and the resulting IC_{50} value was 134.56 ppm. The highest antioxidant activity in the aqueous extract of kawista leaves was 66.12%; 89.51%; and 94.41% (Nachimuthu *et al.*, 2014).

Based on study of Patil *et al.* (2012), 200 ppm extract concentration, the antioxidant activity was measured in the following ways: leaves (57.34 g/mL), stems (55.94 g/mL), fruit skins (55.94 g/mL), and fruit flesh (40.55 g/mL); at 400 ppm extract concentration, the values were as follows: leaves (67.10 g/mL), stems (79.02 g/mL), fruit skins (67.83 g/mL), and fruit flesh (51.04 g/mL); at 600 ppm extract concentrations, the values were as follows: leaves (95.80 g/mL), stems (82.51 g/mL), and fruit skins (67.83 g/mL).

Based on the article obtained in the study “Phytochemical investigation and *in vitro* antioxidant activity of extracts from leaves of *L. acidissima* Linn. (Rutaceae),” compounds that have antioxidant activity found in kawista leaves are flavonoids and flavonols (Attarde *et al*., 2011). Based on the principle of "like dissolves like," secondary metabolite compounds can be separated from plant parts using solvents with appropriate polarity levels to achieve higher biochemical activity. The separation carried out by previous researchers reached the extraction stage, so a more specific separation of kawista leaves has never been conducted. Therefore, a separation process is necessary until active compounds that provide the most optimal antioxidant activity are identified. A fractionation process is needed to obtain active compounds as antioxidants from kawista leaves. This study aimed to determine the antioxidant activity of kawista leaf fractions using DPPH method, and to determine the active compounds in the highest antioxidant agents of kawista leaves fracton.

2. Method

2.1. Tools and materials

The tools used in this research are maceration equipment consisting of a maceration vessel and a beaker glass (Herma). Another tool used in this research is a UV lamp (Recent RCC), a set of UV-VIS spectrophotometry equipment from Shimadzu 1700, an analytical balance (Fujitsu), an oven (Binder ED), a mesh sieve 40, a blender (Phillips), a rotary evaporator (B ONE RE 2000), and silica GF 254 nm. (Merck).

The material used in this research is kawista leaf powder produced from Dresi Kulon Village, Kaliori District, Rembang Regency. The chemical materials used in this research were 96% ethanol p.a (Sigma), DPPH (Sigma), methanol p.a (Sigma), aquadest (Bratachem), n-hexane (Bratachem), ethyl acetate (Bratachem), n-butanol (Bratachem), vitamin C (Sigma), ether (Bratachem), acetic acid (Bratachem), sulfanilic acid (Nitrokimia), formaldehyde (Bratachem), ammonia (Bratachem), chloroform (Bratachem), amyl alcohol (Bratachem), NaOH (Sigma), $NaNO_2$ (Bratachem), HCl (Bratachem), sulfuric acid (Bratachem), vanillin (Bratachem), anisaldehyde (Bratachem), Dragendorff's reagent (Bratachem), Meyer's reagent (Bratachem), and $FeCl_3$ (Bratachem).

2.2. Method and result analysis

The selected extract that continued the purifying process was the methanol extract, which will henceforth be referred to as the concentrated extract of kawista leaves. It has the best AAI value among the three extracts that have been tested for DPPH antioxidant activity using a spectrophotometer UV-Vis (Azizah *et al*., 2024).

2.2.1. LLE Method

Methanol extract of kawista leaves was re-extracted on a large scale until a selected concentrated extract was obtained for liquid-liquid extraction process, which includes the separation of the concentrated extract from the leaves first, then partitioned into n-hexane, ethyl acetate, and water. The extract was dissolved in a 1:1 mixture of n-hexane and distilled water. After shaking for 10–15 minutes in the separating funnel, the water will settle at the bottom and n-hexane will rise to the top.

2.2.3. Vacuum liquid chromatography method

As much as 5 grams of the selected sample kawista leaf was based on the activity test above, then add it to approximately 5 grams of the stationary phase of the silica gel column and stir until it forms a powder. Then, it was re-fractionated using vacuum column chromatography with silica gel as the stationary phase and a solvent mixture as the mobile phase. This was done to look at the

fractions that separated the best in the TLC analysis, starting with 50 mL (Gritter *et al*., 1991). From non-polar (n-hexane) to polar (ethyl acetate) to non-polar (methanol), the elution solvent increases in polarity. To ensure maximum density, the sample was deposited at the top of the column and evenly spread before the filter paper was placed on top and the vacuum device is activated. The column was completely left blank after each collection.

2.2.4. Study of antioxidant activity

DPPH solution was prepared by adding 50 mg of DPPH powder into a 50.0 mL volumetric flask, p.a. methanol, resulting in a concentration of 1000 ppm. The sample was diluted 10 times using a 5.0 mL pipette and p.a. methanol to reach a concentration of 100 ppm up to the mark of a 50.0 mL volumetric flask. The determination of antioxidant activity was carried out by adding 2 mL of 100 ppm DPPH solution into a test tube, followed by the addition of 2 mL of each sample solution: each concentration of 60, 80, 100, 120, and 140 ppm. Next, the mixture was homogenized with a vortex for 10 seconds and allowed to stand according to the operating time of each test solution in a dark place. The solution's absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength. Measurements of the absorbance of the control solution (DPPH) and the standard of vitamin C were also conducted (Erika *et al*., 2014). The absorbance value of kawista leaves was then used to calculate the percentage of free radical scavenging activity using the formula (inhibition percentage):

$$\frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}}\ x\ 100\%$$

After obtaining the inhibition percentage from each replication, a linear regression calculation was performed between the sample concentration (ppm) (X) and the DPPH inhibition percentage (%) (Y), resulting in the equation: Y = BX + A. The IC_{50} value was obtained from the x value after substituting y with 50 by inserting the values of B and A. The same calculation was also performed on the vitamin C standard. Once the IC_{50} value was obtained, it was then converted into the AAI (Antioxidant Activity Index) value, which was used to determine the antioxidant activity index using the formula (AAI):

$$\frac{\text{DPPH concentration (ppm)}}{\text{IC50 (ppm)}}$$

2.2.5. Study of phytochemicals

The identification of compounds conducted includes qualitative antioxidant tests, flavonoid TLC tests, alkaloid TLC tests, saponin TLC tests, and tannin TLC tests. The stationary phase used is silica gel GF254. The mobile phase for the flavonoid test was n-butanol:acetic acid:water (4:1:5), and

the reagent used was ammonia vapor. The mobile phase for the alkaloid test was methanol:chloroform (0.5:9.5), and the reagent used was Dragendorff's reagent. The mobile phase for the saponin test was chloroform:methanol:water (6:3:1), and the reagent used was H_2SO_4 – anisaldehyde. The mobile phase for the tannin test was ethyl acetate:methanol:water (100:13.5:10), and the resulting chromatogram was detected with visible light, UV254 light, and UV 366 light, then marked and the Rf values were calculated (Harborne, 1987). Then the highest fraction had identified of compounds using FTIR aims to determine the functional groups of a compound that produce characteristic bands of that compound, if the compounds had characterized be flavonoids so was carried out through analysis using spectrophotometer UV-Vis.

3. Result and discussion

The three solvents used in this study were n-hexane, which has a polarity index of 0, meaning it is non-polar, ethyl acetate, which has a polarity index of 4.4; indicating that ethyl acetate could dissolve semi-polar compounds; and water, which had a polarity index of 9.0; meaning it was very polar and could attract the polar compounds. The highest yield resulted from the water fraction, followed by the n-hexane and ethyl acetate fractions.

Tabel 1. Yield value of kawista fraction

Methanol extract (gram)	Solvent	Result (gram)	Yield (%)
38.1000	n-hexane	10.1320	26.59
	Ehtyl Acetate	5.8033	15.23
	Water	12.9670	34.03

The results in **Table 1** show that the yield of ethyl acetate solvent is lower compared to other solvents, due to the high levels of bioactive compounds present in the ethyl acetate sample. The antioxidant activity quantitatively of the kawista leaf purified extract using the methods above (spectrophotometer UV-Vis), maximum wavelength obtained is 515.9 nm with an absorbance of 0.733 (within the range of 0.2 - 0.8).

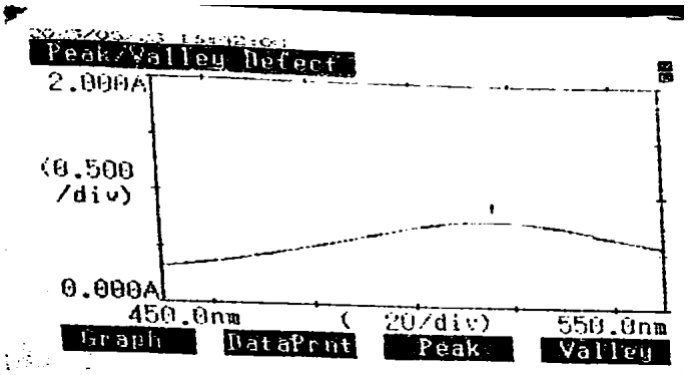

Figure 1. Maximum wavelength of DPPH solution

Furthermore, the operating time obtained from the vitamin C standard is at the 30th minute, and for the n-hexane, ethyl acetate, and water fractions, the operating times obtained are at the 28th, 26th, and 29th minutes, respectively. The AAI value data for the kawista leaf purified extract can be seen at **Figure 2**.

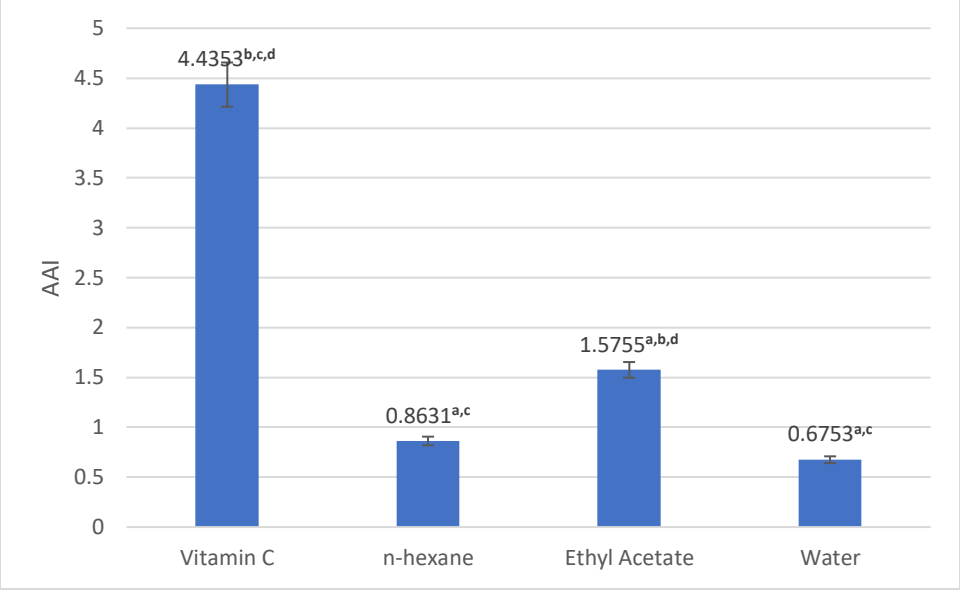

Figure 2. Test antioxidant activity of purified extract

Description:
a. significantly different ($p < 0.05$) from vitamin C
b. significantly different ($p < 0.05$) from the n-hexane sample
c. significantly different ($p < 0.05$) from the ethyl acetate sample
d. significantly different ($p < 0.05$) from the water sample

The selection of the sample that will continue with the separation using VLC is based on the best AAI values. As seen from the statistical test, the aqueous sample and the n-hexane sample did not show significant differences from each other. Therefore, if only one solvent was chosen to proceed with the VLC separation, it was highly likely that there will be no difference in antioxidant activity.

The selected ethyl acetate sample was further processed using VLC to separate the active compounds from kawista leaves that have the best antioxidant activity. The choice of separation method using VLC was due to the fact that the sample can migrate quickly in both the stationary and mobile phases under vacuum conditions, thus enabling the production of compounds more effectively (Mutmainnah *et al.*, 2017). The ethyl acetate sample was first impregnated by carefully weighing 5 grams of the ethyl acetate fraction from the kawista leaves, then adding it to approximately 5 grams of the stationary phase of silica gel column and the mobile phase of n-hexane, ethyl acetate, and methanol (Rahimah *et al*., 2013).

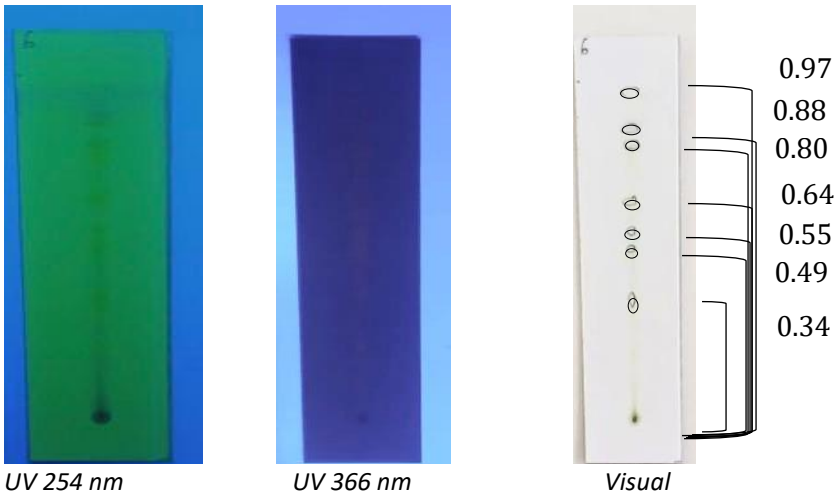

Figure 3. Chromatogram profile of methanol extract optimization
[the stationary phase: silica gel GF 254; mobile phase: n-hexane:ethyl acetate (v/v) as (7:3)]

The result from the VLC separation was collected in glass bottles (11 eluates). The separation of several fractions could be observed from the appearance of color differences in the compounds that elute along with the mobile phase, where the colors range from the darkest to nearly clear, and the clear color resembling that of the mobile phase is considered to not contain significant active compounds. Re-analyze using TLC with a stationary phase of silica gel GF254 and an optimal mobile phase ratio of n-hexane:ethyl acetate:methanol on the methanol extract. Among the various mobile phases used, the sixth mobile phase, which was n-hexane:ethyl acetate (v/v) (7:3), was the optimal mobile phase for TLC results from VLC. This was because the spots provide a fairly good separation pattern on the methanol extract, as seen in **Figure 3**.

Then, 11 eluates were eluted using TLC with the optimal mobile phase of n-hexane:ethyl acetate (v/v) (7:3). If the same spot pattern is observed under UV light, they can be combined to obtain a simpler fraction result. The results of the chromatogram pattern of the fraction can be seen in **Table 2**.

Table 2. Result of chromatogram pattern of kawista fraction

Eluates	Eluents	Total	Total of spots	Rf	Fraction code
1st	n-hexane	100% (v/v) 100 mL	0	-	F1
2nd	n- hexane : ethyl acetate	8 : 2 (v/v) 100 mL	0	-	F1
3rd	n- hexane : ethyl acetate	6 : 4 (v/v) 100 mL	0	-	F1
4th	n- hexane : ethyl acetate	4 : 6 (v/v) 100 mL	4	0.1 0.2 0.38 0.61	F2
5th	n- hexane : ethyl acetate	2 : 8 (v/v) 100 mL	4	0.1 0.2 0.38 0.61	F2
6th	ethyl acetate	100% (v/v) 100 mL	4	0.1	F2

Eluates	Eluents	Total	Total of spots	Rf	Fraction code
				0.2 0.38 0.61	
7th	ethyl acetate : methanol	8 : 2 (v/v) 100 mL	2	0.1 0.2 0.38	F3
8th	ethyl acetate : methanol	6 : 4 (v/v) 100 mL	2	0.1 0.2 0.38	F3
9th	ethyl acetate : methanol	4 : 6 (v/v) 100 mL	2	0.1 0.2	F4
10th	ethyl acetate : methanol	2 : 8 (v/v) 100 mL	2	0.1 0.2	F4
11th	methanol	100% (v/v) 100 mL	2	0.1 0.2	F4

Furthermore, the four combined fractions were subjected to quantitative antioxidant activity testing using the same methods and procedures as the antioxidant tests of the kawista leaves. The maximum wavelength obtained is 514.8 nm with an absorbance of 0.785 (within the range of 0.2 - 0.8). Next, the operating times obtained from F1, F2, F3, and F4 were at the 25th, 27th, and 29th minute, respectively. The results can be seen at **Table 3**.

Based on the results of the statistical test, it can be concluded that the vitamin C group does not have a significant difference compared to the F2 group of kawista leaves, indicating that the activity of the F2 group is equivalent to that of the positive control. The identification results of the chemical compounds in the ethyl acetate fraction of the kawista leaves only positively indicated the presence of flavonoids and tannins. Therefore, the researchers concluded that the compounds likely present in subfraction F2 are these two compounds.

Table 3. Test antioxidant activity of kawista fraction

No	Sample	IC_{50} (ppm) ± SD	AAI ± SD
1	F1	66.6703 ± 4.7025	1.5049 ± 0.1068
2	F2	29.7671 ± 2.9362	3.3808 ± 0.3255
3	F3	48.3196 ± 5.4403	2.0877 ± 0.2426
4	F4	123.4360 ± 1.3663	0.8102 ± 0.0090
5	Vitamin C	22.6424 ± 1.7887	4.4353 ± 0.3576

Separation of the F2 fraction using preparative TLC with silica gel 60 F254 stationary phase, with the spots elongated along the TLC plate. Then, a positive band containing flavonoids was produced with the mobile phase of n-butanol: ethyl acetate: water, and the band was extracted in 3 mL of methanol solvent. After the solvent evaporated, the formed crystals were further analyzed using UV-Vis and FTIR spectrophotometers.

The FTIR spectrum produced from the active fraction of kawista leaves (F2) is shown in **Figure 4**.

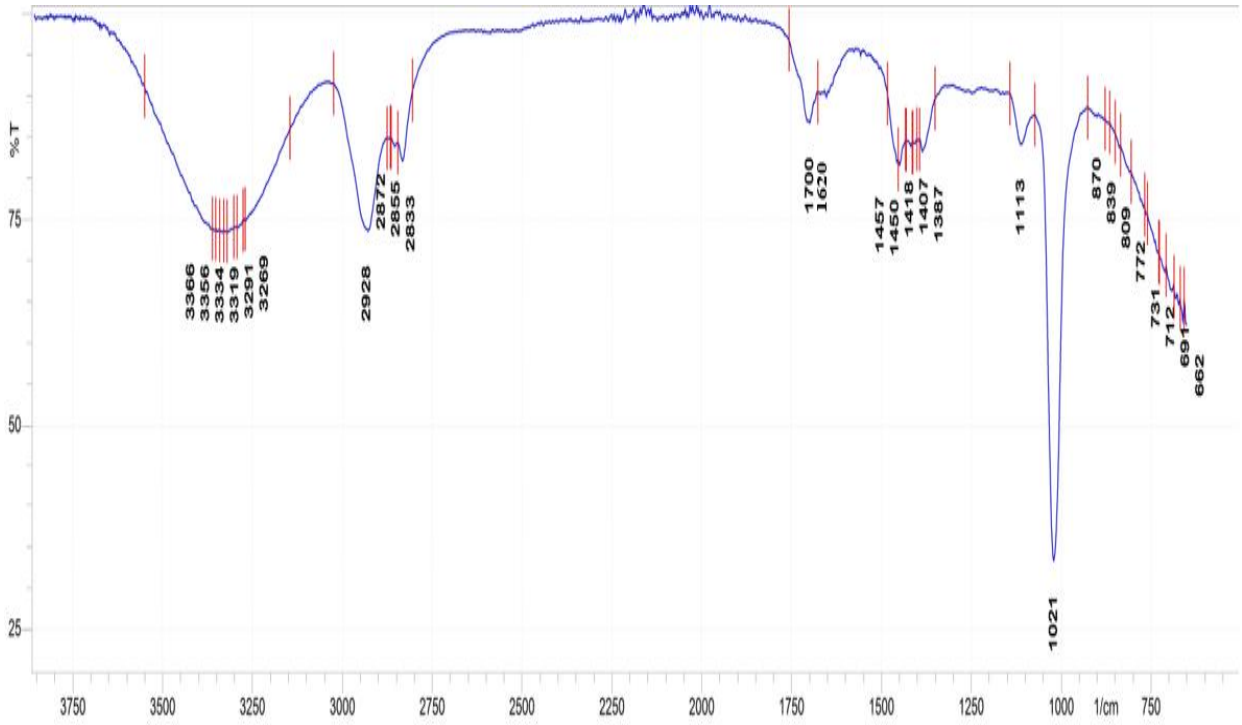

Figure 4. FTIR Spectrum of F2

The FTIR spectrum in **Figure 4** showed the presence of characteristic vibrational absorptions that identify several functional groups present in the structure of flavonoid bonds. The sharp absorption peak at the wave number 1021 cm^{-1} indicates the presence of the stretching vibration of the C–O ether bond.

The characteristics that support the presence of aromatic rings are indicated by several vibrational absorptions, including those that show the stretching vibration =C–H aromatic at wavenumbers to the left of 3000 cm^{-1} and the bending vibration =C–H aromatic at wavenumbers 870 – 691 cm^{-1}, as well as the vibrational absorptions in the wavenumber region of 1600 cm^{-1} and 1457 cm-1, which are the stretching vibrations C=C of the aromatic ring as a chromophore group typical of flavonoids in a conjugated bonding system. The absorption band at the wavenumber of 1700 cm^{-1} indicates the presence of C=O stretching vibrations of ketones, and it is close to the absorption band of C=C stretching vibrations of alkenes around the wavenumber of 1620 cm^{-1}.

Based on the interpretation of the wavelength shift data obtained, the flavonoid compound that may belong to subfraction F2 is classified as a flavonol, with the possibility of hydroxyl groups present at C-7 and C-4'. The wavelength shift in fraction F2 allows us to conclude the proposed structure of the flavanon compound as shown in **Figure 4**.

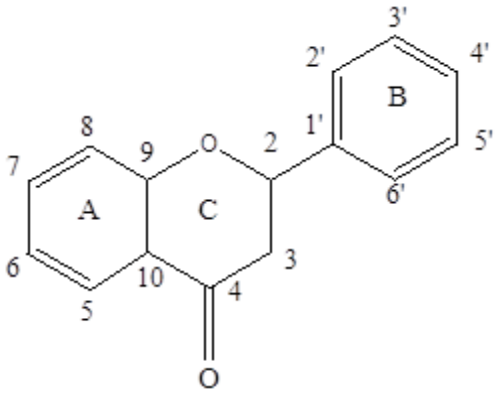

Figure 5. The structural hypothesis of flavonoid compounds in fraction F2 (flavanon)

4. Conclusion

Active compounds from kawista leaves fraction (F2) as the fraction using n-hexane and ethyl acetate solvents had the highest antioxidant agent (AAI = 3.3808 $\pm$ 0.3255) equivalent to the positive control (Vitamin C) with possibility flavonoid compound from F2 belong to the flavanon group.

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An analysis of the cost-effectiveness of filgrastim versus lenograstim in colorectal cancer patients with FOLFOX chemotherapy regimen

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Abstract

Background: Granulocyte colony-stimulating factor (GCSF) is a primary regulator of the granulopoiesis process, which mobilizes stem cells from the bone marrow to the blood vessels. Filgrastim and lenograstim are the types of GCSF that have been widely used.

Objective: This research aims to study the comparative therapeutic outcomes and cost-effectiveness of filgrastim and lenograstim therapies in colorectal cancer patients undergoing chemotherapy with the FOLFOX regimen.

Method: This research was conducted at the pharmacy installation of the Dr. Kariadi Central General Hospital (RSUP Dr. Kariadi) from December 2023 to January 2024. It is observational research with a retrospective pre-posttest cohort study design that evaluates the comparative effectiveness and costs of filgrastim and lenograstim therapies in patients with neutropenia based on an increase in white blood cells (WBC) and absolute neutrophil count (ANC) scores of patients. The data was analyzed using the unpaired comparative analysis method and using the cost-effectiveness analysis (CEA) method to determine the cost-effectiveness. The observations were carried out twice, before and after administering the GCSF therapy. The samples consisted of 25 patients divided into two treatment groups.

Results: The average scores of WBC and ANC levels in 15 patients who received filgrastim therapy were 2000 cells/mm^3 and 666 cells/mm^3. At the same time, the WBC and ANC levels in 10 patients who received lenograstim therapy were 1980 cells/mm^3 and 449 cells/mm^3. After administering the GCSF therapy, there was a significant increase in WBC and ANC levels ($p<0.05$) in each group. Still, there was no significant difference in the increase in WBC and ANC between the groups receiving filgrastim and lenograstim ($p>0.05$). The CEA analysis results showed that an increase of 1 cell/ml of WBC score cost Rp24.2 for filgrastim and Rp347 for lenograstim. In contrast, an increase of 1 cell/ml of ANC score cost Rp111.8 for filgrastim and Rp1572.5 for lenograstim.

Conclusion: This research concludes that filgrastim is as effective as lenograstim, yet filgrastim is considered more cost-effective than lenograstim.

Keywords: GCSF, filgrastim, lenograstim, neutropenia, cost-effectiveness analysis

1. Introduction

Chemotherapy is one of the cancer treatments. It can cause various side effects, even unexpected toxic effects. As many as 5–15% of deaths occur due to the toxic effects of chemotherapy. One of the common toxic effects of cancer chemotherapy is bone marrow suppression, which causes myelosuppressive effects (Bracci *et al*., 2014).

Neutropenia is a condition in which the number of neutrophils in the blood decreases, less than 500 cells/mm^3 or less than 1000 cells/mm^3. Neutropenia can occur due to impaired formation and shift of neutrophils to tissue, as well as increased destruction of neutrophils in the circulation. Disruption of neutrophil formation can occur due to infiltration of malignant cells and the myelosuppressive effects of chemotherapy drugs (Pilatova *et al.*, 2018). The severity of neutropenia depends on the Absolute Neutrophil Count (ANC), a measure of the

number of neutrophils in the blood. Severe neutropenia occurs when ANC <500 cells/mm^3 (American Academy of Allergy, 2023).

Neutropenia can occur in cases of hematological malignancies and solid cancers. Around 20-40% of neutropenia occurs in solid cancers. The majority of neutropenic episodes occur in the first cycle of chemotherapy in breast cancer as much as 71%, lymphoma cancer as much as 70%, colorectal cancer 53%, ovarian cancer as much as 46%, and lung cancer as much as 60% (Smith *et al*., 2015). The hematopoietic growth stimulating factor is a cytokine that regulates the proliferation, differentiation, and function of hematopoietic cells. There are two forms of hematopoietic growth-stimulating factors: granulocyte colony-stimulating factor (GCSF) and granulocyte-macrophage colony-stimulating factor (GMCSF). Examples of GCSF are lenograstim, filgrastim, and pegylated filgrastim. In contrast, an example of GM-CSF is sargramostim. Both types have been widely studied in cancer patients at risk of neutropenia as prevention or therapy for febrile neutropenia (Pilatova *et al*., 2018).

Systematic review and meta-analysis studies state that mortality is reduced in patients who receive GCSF after chemotherapy. However, in these studies, GCSF (filgrastim) was not used as a therapy for neutropenia but was used as primary prophylaxis (Mitchell *et al*., 2016). According to ESMO (European Society for Medical Oncology) patients with neutropenia can be treated with GCSF, which can increase neutrophils, reduce the incidence of febrile neutropenia, duration of neutropenia, length of treatment, reduce the number of morbidity and mortality rates in cancer patients (Klastersky *et al.*, 2016).

Several previous studies have shown different results, making the use of GCSF as a therapy still controversial. In addition, Wang *et al.*'s study found that the use of long-acting GCSF agents was more cost-effective than short-acting GCSF agents (Wang *et al*., 2023). However, the widespread use of GCSF agents is still limited because it is associated with high costs or prices (Klastersky *et al*., 2016). This research aims to compare the outcomes of WBC and ANC increases and the cost effectiveness of using filgrastim and lenograstim in colorectal cancer patients with neutropenia who would undergo FOLFOX chemotherapy.

2. Method

2.1. Research design and sampling techniques

This research is a retrospective cohort pre-posttest study in which WBC and ANC observations in each group were carried out twice, i.e., baseline data and post-GCSF therapy data.

This research has been approved by the research ethics committee of Dr. Kariadi Central General Hospital (RSUP Dr. Kariadi), with the number 16139/EC/KEPK-RSDK/2024.

This research was conducted at the pharmacy installation of RSUP Dr. Kariadi during the research period from December 2023 to January 2024. The sampling in this research was carried out using the proportionate stratified random sampling technique, where each treatment group sample was selected randomly based on a simple lottery. The number of samples in this research was determined using the total sampling method. The sample criteria in this research are as follows:

1. Inclusion criteria
   a. Adult patients aged 17–60 years.
   b. Patients diagnosed with colorectal cancer and undergoing FOLFOX chemotherapy regimen.
   c. Patients having neutropenia (WBC levels <4000 cells/mm^3 and ANC <1500 cells/mm^3)
2. Exclusion criteria
   Patients having infections that would affect WBC and ANC scores.
3. Drop-out criteria
   a. Patients having hypersensitivity reactions to filgrastim or lenograstim.
   b. Patients who died during the research period.
   c. Patients who returned home at their request without any blood tests after filgrastim or lenograstim therapy.

2.2. Data analysis

To determine the effectiveness of therapy, a comparative hypothesis test was conducted on the patient's WBC and ANC profiles before and after GCSF therapy was administered to each treatment group. The comparative test used was the paired t-test if the data distribution was normal ($p>0.05$). However, the Wilcoxon test was used if the data distribution was abnormal ($p<0.05$).

An unpaired comparative hypothesis test (independent t-test) was conducted to determine whether there was a difference between changes in the patient's WBC and ANC profiles after GCSF therapy was administered between the filgrastim and lenograstim groups. The comparative test used was the independent t-test if the data distribution was normal ($p>0.05$). Still, the Wilcoxon test was used if the data distribution was abnormal ($p<0.05$) (Dahlan, 2009).

Furthermore, to compare cost-effectiveness, a CEA—ACER (average cost-effectiveness ratio) analysis was carried out by measuring the average direct costs, i.e., the total cost of GCSF, compared to the average outcome of increasing WBC and ANC cell levels per mm^3.

3. Results and discussion

During the data collection period from December 2023 to January 2024, at RSUP Dr. Kariadi, 25 patients met the inclusion criteria. Of the 25 samples, 15 patients received filgrastim therapy, and 10 patients received lenograstim therapy. **Table 1** showed a picture of patients having neutropenia based on sex, where the percentage of female patients is more dominant, namely 14 patients (54.94%) compared to 11 male patients (47.06%). Of the 25 patients, there are 19 patients (76%) aged 18–59 years, while six patients (24%) were >60 years old, and no patients are <18 years old.

In this research, all patients (100%) experience neutropenia with a WBC score <4000 cells/ml, while 13 patients (52%) experience severe neutropenia with an ANC score <500 cells/ml, and 12 patients (48%) experienced mild-moderate neutropenia.

Table 1. Patient characteristics

Patient characteristics		Total	Percentage
Sex	Male	11	44
	Female	14	56
Age	< 18 years old	0	0
	18–59 years old	19	76
	> 59 years old	6	24
Total WBC	> 4000 cells/ml	0	0
	< 4000 cells/ml	25	100
Total ANC	Mild (1000–1500 cells/ml)	3	12
	Moderate (500–1000 cells/ml)	9	36
	Severe (<500 cells/ml)	13	52
Colorectal cancer stages	Stadium II	0	0
	Stadium III	8	32
	Stadium IVa	10	40
	Stadium IVb	7	28
	Stadium IV c	0	0
FOLFOX Chemotherapy Cycle	Cycle 1	0	0
	Cycle 2	1	4
	Cycle 3	1	4
	Cycle 4	5	20
	Cycle 5	3	12
	Cycle 6	2	8
	Cycle 7	3	12
	Cycle 8	2	8
	Cycle 9	1	4
	Cycle 10	1	4
	Cycle 11	3	12

Patient characteristics		Total	Percentage
	Cycle 12	3	12
Nutritional Status	Normal	22	88
	Malnutrition	3	12

Table 2 showed the WBC and ANC scores in the group of patients receiving filgrastim therapy. Before the filgrastim therapy, all groups of patients (100%) had WBC scores <4000 cells/ml, while based on the ANC score, there were seven patients (47%) with ANC scores <500 cells/ml, five patients (33%) with ANC scores of 500–1000 cells/ml, and three patients (20%) with ANC scores of 1000–1500 cells/ml. The WBC and ANC scores of patients after receiving the filgrastim therapy all had increased, but there were six patients whose ANC scores were still below the normal score after the lenograstim therapy.

Table 2 WBC and ANC score profile of patients with filgrastim therapy

Before the therapy						After the therapy					
WBC Score	Σ	%	ANC Score	Σ	%	WBC Score	Σ	%	ANC Score	Σ	%
< 4000	15	100	> 1500	0	0	< 4000	4	27	> 1500	9	60
			1000–1500	3	20				1000–1500	4	28
			500–1000	5	33				500–1000	1	6
> 4000	0	0				> 4000	11	73			
			< 500	7	47				< 500	1	6
Total	15	100		15	100		15	100		15	100

Table 3 showed the WBC and ANC scores in the group of patients receiving lenograstim therapy. Before the lenograstim therapy, there were ten patients (100%) with a WBC score of <4000 cells/ml, while based on the ANC score, there were six patients (60%) with an ANC score of <500 cells/ml, four patients (40%) with an ANC value of 500–1000 cells/ml, and no patients with an ANC score of 1000–1500 cells/ml or more than 1500 cells/ml. The WBC and ANC scores of patients after receiving the lenograstim therapy were all above the normal score. However, there were four patients whose ANC scores were still below the normal score after the lenograstim therapy.

In general, both groups of patients with filgrastim and lenograstim therapies experienced an increase in WBC and ANC scores after the therapy was administered. The data obtained were tested for data normality first using the Shapiro–Wilk method and obtained a test of WBC and ANC data distribution in each normal group. To determine whether there was a difference between changes in WBC and ANC scores in each treatment group after receiving the therapy, a statistical test was carried out using the Paired T-test. **Table 4** below showed the analysis results of the two parameters.

Table 3 WBC and ANC scores profile of patients with lenograstim therapy.

Before the therapy						After the therapy					
WBC Score	Σ	%	ANC Score	Σ	%	WBC Score	Σ	%	ANC Score	Σ	%
< 4000	10	100	> 1500	0	0	< 4000	2	20	> 1500	6	60
			1000–1500	0	0				1000–1500	3	30
> 4000	0	0	500–1000	4	40	> 4000	8	80	500–1000	0	0
			< 500	6	60				< 500	1	10
Total	10	100		10	100		10	100		10	100

Table 4 Statistical analysis results of WBC and ANC parameters

Parameter	Filgrastim			Lenograstim		
	Before	After	Sig (p)	Before	After	Sig (p)
WBC Score	2000± 879.1	9553 ± 1962.7	0.002	1980 ± 1003.1	6990 ± 1410	0.003
ANC Score	666 ± 462.1	2304 ± 386	0.002	449 ± 244.8	1556.2 ± 1302.2	0.001

To determine whether there is a difference in the increase in WBC and ANC levels in patients receiving filgrastim and lenograstim therapies, a statistical analysis was conducted on changes in WBC and ANC scores using the independent t-test method. **Table 5** below showed the analysis results of changes in the scores of the two variables.

Table 5 Statistical analysis results of changes in WBC and ANC scores

Parameter	Filgrastim	Lenograstim	Sig (p)
Δ WBC	7553.3 ± 2040.8	5010	0.359
Δ ANC	1637 ± 439.5	1107.2 ± 230	0.365

A total of 15 patients with neutropenia received the filgrastim therapy. Before receiving the filgrastim therapy, the baseline WBC and ANC values of all patients are below normal scores. After the filgrastim therapy, there is a significant increase in WBC and ANC levels ($p<0.05$).

Neutrophils are the main target of GCSF. Exogenous GCSF increases the number of peripheral blood neutrophils in humans by stimulating the expansion of neutrophil precursors and increasing the maturation rate and release of neutrophils into the circulation. GCSF stimulates neutrophil production and mobilizes bone marrow neutrophils and stem cells into the blood. GCSF has also improved the cellular functions of mature neutrophils, including adhesion and phagocytosis (Scotte *et al*., 2024). GCSF can reduce the duration of treatment due to neutropenia. According to study conducted by Utomo *et al*., that the duration of treatment for cancer patients due to febrile neutropenia who were given GCSF decreased by 3 days compared to placebo, namely 5 days (Utomo *et al*., 2020).

Filgrastim is a GCSF analog that works by stimulating proliferation and differentiation by interacting specifically with receptors found on various myeloid stem cells, which will develop into neutrophils. In addition, filgrastim also activates the phagocytic process of mature

neutrophils, prolongs the duration of neutrophils in the blood and directs hematopoietic stem cells to increase their concentration in the peripheral circulation (Scotte *et al.*, 2024).

GCSF consists of a non-glycosylated recombinant form synthesized from *Escherichia coli* (filgrastim) and a glycosylated form synthesized from Chinese hamster ovary cells (lenograstim). The glycosylation process on filgrastim compounds causes modification of chemical properties, such as molecular rigidity, pH, temperature, and higher elastase, resulting in a longer plasma half-life. In addition, the glycosylation process will reduce aggregate formation and increase receptor affinity, leading to increased bioavailability and molecular activity (Ria *et al*., 2010).

A total of 10 neutropenia patients in this study received the lenograstim therapy. Before receiving the lenograstim therapy, the baseline WBC and ANC scores of all patients were below normal values. After undergoing the lenograstim therapy for two days, there was a significant increase in WBC and ANC levels ($p<0.05$).

Various studies have been conducted to prove the effectiveness of lenograstim in patients experiencing febrile neutropenia. These studies show that lenograstim is excellent at reducing the incidence of febrile neutropenia (Cooper *et al.*, 2011). In line with previous studies, the administration of lenograstim in this research showed a significant increase in WBC and neutrophil levels.

Another factor that affects neutropenia is nutritional deficiency (Budiana & Febiani, 2017). Based on the results of the nutritionist assessment, patients having neutropenia after receiving the lenograstim therapy were deficient. Malignancy patients will have nutritional deficiencies caused by several factors, such as lack of food intake due to the influence of side effects of chemotherapy drugs and malabsorption of nutrients in the gastrointestinal tract. A study showed that chemotherapy patients would experience several nutritional deficiencies, such as vitamin A and retinol, vitamin E, vitamin C, beta carotene, selenium, zinc, and B vitamins (Garófolo, 2013).

Analysis of changes in WBC and ANC values was carried out in each treatment group to assess the comparative achievement of filgrastim and lenograstim therapies. The results of statistical tests using the independent t-test test showed no significant difference ($p>0.05$) between changes in WBC values ($p = 0.359$) and ANC ($p = 0.365$) in the group of patients receiving the filgrastim therapy and the group of patients receiving the lenogastim therapy. The results in this research are different from the results of a study by Orciuolo *et al.* in 2011, which stated that episodes of febrile neutropenia were lower in the group of patients who received the lenograstim therapy when compared with the filgrastim. However, a study by Uddin *et al*. (2015)

found that filgrastim biosimilar was as effective as lenograstim for HSCT (hematopoietic stem-cell transplant) in patients with lymphoma. However, mobilization with filgrastim biosimilar was superior to lenograstim in younger MM (multiple myeloma) patients (Uddin *et al*., 2015).

Glycosylation in GCSF will cause changes in its pharmacokinetic profile, thereby increasing its bioavailability and molecular activity. Other studies have shown that glycosylation in GCSF causes inactivation of the elastase enzyme, thereby prolonging the duration of action of GCSF and increasing its effectiveness (Ria *et al.*, 2010). In addition, there was experimental evidence that the bond between sugar and protein (glycosylation) plays a critical role in the activity of neutrophils. Neutrophils mobilized by lenograstim show more mature expression in terms of recognition, adhesion, phagocytosis, and interaction with immunoglobulins (Ria *et al.*, 2010).

The similarity in therapeutic achievement in the filgrastim and lenograstim groups can be caused by various factors, such as differences in cancer stage, chemotherapy cycles received by patients, and nutritional status of patients. Therefore, further research is needed using a larger number of samples, with the same type of disease and severity and the same chemotherapy regimen between treatment groups.

Furthermore, a CEA analysis was conducted to compare cost-effectiveness. A study conducted by Tseng *et al.* suggests that primary prophylaxis with either a short or long-acting GCSF could be considered cost-effective in patients with breast cancer receiving chemotherapies (Tseng *et al*., 2024). A study conducted by Wang *et al.* found that the use of long-acting GCSF agents is more cost-effective than short-acting GCSF agents (Wang *et al*., 2023). However, the widespread use of GCSF agents is still limited because it is associated with high costs or prices (Klastersky *et al*., 2016).

The results of the cost-effectiveness analysis of the two regimens are shown in **Table 6** below. A cost effectiveness analysis was conducted on the total cost components of GCSF given to each treatment group compared to the increase in WBC and ANC parameter values. The analysis method used was CEA because it aims to compare two drugs used for the same indication but with different costs and effectiveness. Assessment of CEA using the ACER method aims to compare the total costs of a program or alternative treatments with clinical outcomes to produce a comparison that represents the costs of each clinical outcome. According to this comparison, we can choose an alternative treatment with lower costs (Lorensia & Doddy, 2016). The ACER value is the cost that needed to increase 1 unit efficacy of the treatment (Citraningtyas

et al., 2018). The cost data used in this research was the total administration of GCSF until the WBC and ANC scores reached normal values (WBC> 4000 cells/ml, ANC> 1500 cells/ml).

The analysis results showed that the use of the filgrastim regimen was considered more cost-effective than the lenograstim. The ACER calculation results in **Table 6** show that, for an increase of 1 cell/ml of WBC value, a cost of Rp24.2 is required for the filgrastim and Rp347 for the lenograstim regimen. Then, for an increase of 1 cell/ml ANC value, it costs Rp111.8 for the filgrastim and Rp1572.5 for the lenograstim regimen. The lower value of ACER and the higher effectiveness mean more cost effective the therapy (Nalang *et al*., 2018). The ICER (incremental cost-effectiveness ratio) calculation was not carried out because the filgrastim and lenograstim regimens were considered equally effective in increasing the patient's WBC and ANC scores.

Table 6 Cost-effectiveness analysis results

Parameter	Filgrastim	Lenograstim	Cost value / outcome (ACER)	
			Filgrastim	Lenograstim
Average total Cost of GCSF	Rp183,143	Rp1,741,051		
Δ WBC	7553.3	5010	Rp24.2	Rp347
Δ ANC	1637	1107.2	Rp111.8	Rp1572.5

This research's limitation is that no baseline data test was conducted because it is preliminary and has a limited number of samples.

4. Conclusion

The results showed that filgrastim and lenograstim therapies were equally effective in increasing patients' WBC and ANC values. However, based on a cost-effectiveness analysis, filgrastim therapy was considered more cost-effective.

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