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Original Research Report

TOXICITY TEST ON THE COMBINATION OF *Caesalpinia sappan* AND *Zingiber officinale* IN *Rattus norvegicus* INDUCED BY COMPLETE FREUND'S ADJUVANT

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ABSTRACT

Sappanwood (*Caesalpinia sappan*) and red ginger (*Zingiber officinale*) are plant species that have been studied for their efficacy in treating inflammation related to rheumatoid arthritis. This study aimed to examine the effects of combining sappanwood and red ginger to determine the potential toxicity of the herbal extracts in medicine. The toxicity testing was carried out in vivo using 32 Wistar strain male white rats (*Rattus norvegicus*). The animal models were divided into eight groups consisting of four rats each. The rats were injected with complete Freund's adjuvant to induce a chronic inflammatory effect. The eight groups consisted of the negative control group, the positive control group, the normal group, and five treatment groups. This study was conducted by observing the animals for toxic symptoms and death to determine the safety of the extracts and drugs. The observation results were analyzed using a one-way analysis of variance ($p<0.05$). The analysis results showed that weight gain and relative organ weight among the groups had no significant differences ($p>0.05$). Microscopic examination of the organ preparations observed under a light microscope revealed no significant changes or adverse effects in rats treated with the extracts or drugs. In conclusion, a combination of sappanwood and red ginger ethanol extracts administered orally has no toxic effect in rats injected with complete Freund's adjuvant.

Keywords: Rheumatoid arthritis; red ginger; sappanwood; toxicity test; good health and well-being

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Highlights:
1. This study determined the toxic effects of combining *Caesalpinia sappan* and *Zingiber officinale* extracts, as people need to be aware of the potential side effects of these common herbal remedies for the treatment of rheumatoid arthritis.
2. The combination of *Caesalpinia sappan* and *Zingiber officinale* extracts is efficacious and non-toxic as an anti-arthritis treatment, hence, the ingredients can be upgraded to standardized herbal medicines and phytopharmaceuticals.

INTRODUCTION

Rheumatoid arthritis (RA) is the most common autoimmune rheumatic disease. It is a chronic inflammatory disease that progresses and causes permanent joint damage. Systemic inflammation in rheumatoid arthritis is strongly associated with several extra-articular comorbidities, including cardiovascular disease, metabolic syndrome, osteoporosis, interstitial lung disease, infection, malignancy, fatigue, depression, and cognitive impairment, thereby increasing morbidity and mortality in rheumatoid arthritis patients (Al-Saadany et al. 2016, Firestein & McInnes 2017, Panagopoulos & Lambrou 2018).

Early diagnosis, management, and timely treatment with nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying antirheumatic drugs (DMARDs) are essential to achieve rapid disease control and prevent further joint damage and disability. It is often challenging to distinguish joint manifestations from the inflammatory process causing arthritis, particularly in patients with early onset. The essential principle of rheumatoid arthritis therapy is to achieve a

therapeutic goal, which is to relieve or at least reduce disease activity (Hidayat et al. 2021). Rheumatoid arthritis drugs can relieve pain and slow the disease progression, but they do not repair tissue damage caused by rheumatoid arthritis. Currently, no known treatment can completely cure rheumatoid arthritis. The use of drugs is also limited because they can cause dangerous side effects such as bleeding in the gastrointestinal tract, cardiovascular complications, impaired kidney function, nausea, dyspepsia, pulmonary toxicity, myelosuppression, liver fibrosis, stomatitis, cirrhosis, immune reactions, and local injection site reactions (Ravikumar 2014, Choudhary et al. 2015). Curative treatment for rheumatoid arthritis is relatively expensive and long-term. It also causes side effects with a high risk of infection. Therefore, 90% of patients are estimated to choose herbal medicines for treating rheumatoid arthritis (Rambod et al. 2018).

Plant species that have been studied for their efficacy in treating inflammation and reducing pain associated with rheumatoid arthritis are sappanwood (*Caesalpinia sappan*) and red ginger (*Zingiber officinale*). Sappanwood contains brazilin and sappanone A, while red ginger contains 6-poradol, 6-shogaol, and 1-dehydro-6-gingerol, which act as anti-arthritis compounds (Jung et al. 2015, Ezzat et al. 2018). There is an increasingly high interest in herbal medicines, which encourages further research on sappanwood and red ginger as rheumatoid arthritis treatments. This study examined the toxic effects of combining sappanwood and red ginger ethanol extracts as a treatment for rheumatoid arthritis. This is essential to provide information and a reference for considering the use of sappanwood and red ginger as effective ingredients in anti-arthritis drugs. Sappandwood and red ginger are anticipated to be upgraded as standardized herbal medicines and phytopharmaceuticals.

MATERIALS AND METHODS

The main materials used in this study were sappanwood and red ginger rhizomes. The sappanwood samples were obtained from Rengganis Jamu Shop, Gresik, Indonesia (7°09'06.7"S 112°39'25.0"E), while the red ginger samples were purchased from Wonokromo Market, Surabaya, Indonesia (7°18'07.8"S 112°44'17.1"E). Other materials used were 96% ethanol, distilled water, complete Freund's adjuvant (CFA) made in the USA by G-Biosciences (38°41'18.251"S 90°22'24.34"E) with batch number 02IJ#200F#201601, diclofenac sodium made in Indonesia by PT Novell Pharmaceutical Laboratories (3°19'4.44"S 115°2'5.28"E) with batch number ALU/02/250, carboxymethylcellulose (CMC) made in Indonesia by PT Gunacipta Multirasa (6°10'41.016"S 106°37'47.999"E) with batch number 3000-01, haematoxylin-eosin stain kit, and 10% neutral buffer formalin (NBF). The test animals used were male Wistar rats (*Rattus norvegicus*) (Barkia et al. 2020). The tools used in this study were syringes produced by Terumo (USA), oral syringes, animal scales, analytical balances, rotary evaporators, volume pipettes, glassware produced by Iwaki (Indonesia), and camera microscopes.

The first method performed in this study was sample extraction. Sappanwood samples were obtained in a dry state. Red ginger rhizomes were washed under running water, sliced thinly, and then dried. The dried samples were ground into a fine powder (Nurhidayah et al. 2022). The samples were extracted by maceration for three, with the fresh solvent being changed every 24 hours. In the maceration process, 1,000 mL of 96% ethanol was used for every 500 g of the samples. Afterwards, the solvent was evaporated using a vacuum rotary evaporator at a temperature of 40°C until it had completely evaporated. The concentrated extract was cooled and stored in a closed container (Aristyanti et al. 2017).

The toxicity testing was carried out in vivo using 32 Wistar strain male white rats (*Rattus norvegicus*) obtained from the Pharmacology Laboratory of Universitas Airlangga, Surabaya, Indonesia. The rats weighed ± 150–200 g and were 3–4 months old. The animal models were acclimatized for 14 days and provided with standard rodent commercial feed and water ad libitum. The categorization of the animal models was performed on the last day of the acclimatization period by weighing and grouping them into eight groups of four in separate cages (Idang et al. 2019).

The in vivo study was conducted through the experiment of administering predetermined doses to the animal models. Signs of toxicity were observed in the percentage of weight gain, relative organ weight, and histopathology of organs. Further monitoring was carried out for 16 days to observe any occurrence of toxic symptoms or death (Pereira et al. 2019). Figure 1 shows the process of toxicity testing. On the first day of the toxicity testing, the rats in seven groups received a subplantar injection of 0.1 mL of complete Freund's adjuvant reagent on their left hind legs to induce rheumatoid arthritis or subchronic inflammatory conditions (Noh et al. 2021).

The toxicity test was observed for 16 days, followed by measuring body weight on day 17. On days 16 to 29, the rats received oral treatment at predetermined doses. Two experimental groups were administered exclusively with sappanwood extract and red ginger extract, respectively. Three groups of rats received a

combination of sappanwood and red ginger extracts in 0.5% carboxymethylcellulose orally with varying doses, i.e., F1 (30 mg/200 g BW of sappanwood extract and 30 mg/200 g BW of red ginger extract), F2 (60 mg/200 g BW of sappanwood extract and 30 mg/200 g BW of red ginger extract), and F3 (30 mg/200 g BW of sappanwood extract and 60 mg/200 g BW of red ginger extract). As a comparison, the three other groups served as the positive control, negative control, and normal groups (Martina et al. 2019). The positive control group received diclofenac sodium in 0.5% carboxymethylcellulose at a dose of 2.7 mg/200 g BW and a combination of 30 mg/200 g BW of each extract (Martina et al., 2019). The negative control group only received 0.5% carboxymethylcellulose. Lastly, the normal group did not receive any treatment (Martina et al. 2019, Amalia et al. 2021).

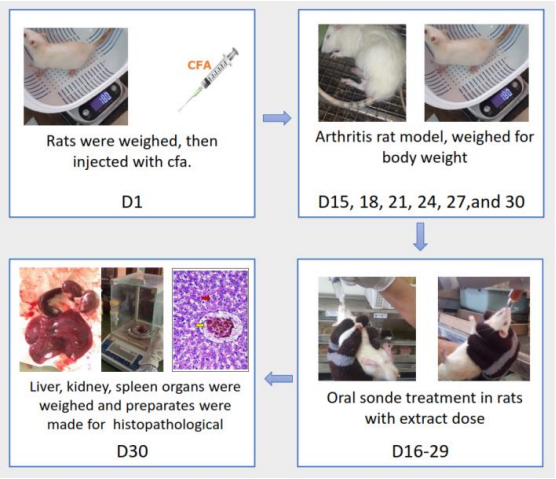

Figure 1. Toxicity testing of extracts using the adjuvant-induced arthritis method.

Body weight gain in the rats was the first sign of toxicity. The rats' body weight was measured on days 1, 15, 18, 21, 24, 27, and 30. Changes in body weight compared to the initial body weight (day 1) were expressed as a percentage using an analytical balance with the following formula (Sunil et al. 2013):

$$\text{Weight gain percentage: } \frac{Wb-Wa}{Wb} x\ 100\%$$

Note:
Wa: body weight before FCA injection (g)
Wb: body weight during the treatment period (g)

The second sign of toxicity was identified according to the assessment of organ weight. Quantitative data on the rats' vital organs (liver, kidney, and spleen) were assessed by weighing the organs of euthanized animals using an analytical balance. The following formula was used for determining the relative organ weight, including the liver, kidney, and spleen (Attanayake et al. 2013):

$$\text{Relative organ weight: } \frac{Wo}{Wb} x\ 100\ \%$$

Note:
Wo: Absolute organ weight (g)

The third sign of toxicity was evaluated using histopathological assessment via hematoxylin-eosin (HE) staining. Observation of the liver, kidney, and spleen tissue was performed using a light microscope with the entire field of view and a magnification of 40X and 400X. The observations showed cell degeneration and necrosis in the kidneys, hydropic and fatty degeneration and necrosis in the hepatocytes of the liver, and bleeding and necrosis in the spleen (Fitmawati et al. 2018, Hidayati et al. 2018, Jannah & Budijastuti 2022).

The data obtained from this study were statistically analyzed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA). Statistical significance among the experimental groups was assessed through one-way analysis of variance (ANOVA) at 5% confidence intervals. The results were expressed as the mean ± standard deviation. A significance value below 0.05 ($p<0.05$) was considered significantly different (Amalia et al. 2021).

RESULTS

On day 15, the plantar condition of the rats' left legs showed that arthritis had developed with symptoms of swelling of the feet, redness of the toes, and changes in the shape of the sole. All of the animals involved in the experiment survived until the day of euthanasia. The body weight of the rats in each group was measured on seven occasions: prior to induction with complete Freund's adjuvant on day 1 and between days 15 and 30, as detailed in Table 1.

Table 2 shows that significant weight gain was observed among rats in the normal group, with a maximum increase of 19.0893%, followed by the sappanwood extract group at 17.3174%. In the groups treated with the combination of extracts (F1, F2, and F3), the percentage of body weight gain on the last day of measurement was 7.7660%, 7.6299%, and 14.4067%, respectively. The results were compared between the treatment and normal groups. There was no statistically significant difference ($p>0.05$) observed in the average body weight changes.

Table 1. Measurement of the rats' body weight in each group.

Samples	Observation						
	D1	D15	D18	D21	D24	D27	D30
Negative	190.2	185.7	191.5	195.0	194.5	195.7	198.5
Positive	185.7	189.2	193.2	193.0	191.7	188.5	190.0
Sappanwood	175.5	200.7	207.2	205.0	208.5	208.7	216.7
Red ginger	224.2	244.5	250.2	253.2	257.7	257.2	261.0
F1	204.0	207.0	213.5	214.2	218.0	219.2	224.5
F2	195.2	196.0	203.2	202.7	205.7	206.5	212.2
F3	178.2	189.2	194.0	198.2	202.5	204.7	208.2
Normal	161.7	188.2	191.5	193.0	195.7	194.7	201.0

Table 2. Body weight increase of the rats in each group (%).

Samples	Observation					
	D15	D18	D21	D24	D27	D30
Nega-tive	-4.3696	-0.6224	1.2982	0.8040	1.4201	2.6338
Positive	1.8766	3.9410	3.7301	2.6847	0.5173	1.3038
Sappan-wood	11.2895	13.9946	12.8912	14.6921	14.2860	17.3174
Red ginger	8.2414	10.0940	11.1907	12.7005	12.6774	13.8319
F1	0.0208	3.2782	5.5910	5.3607	5.8877	7.7660
F2	0.4330	4.0034	3.7055	4.9606	5.1904	7.6299
F3	5.4862	7.7915	9.9437	11.8181	12.8660	14.4067
Normal	13.9973	15.4441	15.9518	17.1107	16.4037	19.0893

The liver, kidney, and spleen weights were measured to observe any potential effects of the extracts, as the organs are the parameters for assessing toxic effects. The statistical test using one-way ANOVA showed that the relative organ weights of the rats' liver, kidney, and spleen were significant ($p>0.05$). As seen in Table 3, measurement of the organ weight between the treatment and control groups showed that there was no significant difference.

The histopathology of vital organs was also observed to determine any potential effects of the extracts. At the end of the 30-day toxicity testing, a histopathological examination was conducted on the liver, kidney, and spleen of the rats in all groups. The histopathological test assessed the presence of cell degeneration and necrosis in the kidney, hepatocytes and necrosis in the liver, as well as bleeding and necrosis in the spleen. There was no difference in the histopathological characteristics of the liver, kidney, and spleen among the rats in each experimental group, as presented in Table 4.

Table 3. Organ weight index of the rats' liver, kidney, and spleen.

Sample	Index of organ weight (%)		
	Liver	Kidney	Spleen
Negative	4.2709	0.0906	0.1627
Positive	4.0321	0.0826	0.1720
Sappanwood	4.1105	0.0694	0.1021
Red ginger	5.0091	0.2299	0.2641
F1	4.5291	0.0803	0.1556
F2	4.1257	0.0366	0.1259
F3	4.4384	0.1116	0.0259
Normal	4.1544	0.1329	0.0833

As shown in Figure 2, the most severe damage to the kidney organs was observed in the negative control group. Rats in the negative control group that did not receive any drugs exhibited 40% organ damage. In the treatment groups, the F3 group experienced the lowest rate of organ damage, at 15%. It was followed by the F1 and F2 groups, with an organ damage rate of 20%. The groups that received sappanwood and red ginger extracts exclusively, however, suffered 25% organ damage.

Table 4. Histopathological examination of the rats' liver, kidney, and spleen.

Samples	Liver	Kidney	Spleen
Negative	Moderate hydropic degeneration (55%)	Moderate cell degeneration (40%)	0
Positive	Mild hydropic degeneration (30%)	Mild cell degeneration (30%)	0
Red ginger	Mild hydropic degeneration (30%)	Mild cell degeneration (25%)	0
Sappanwood	Mild hydropic degeneration (30%)	Mild cell degeneration (25%)	0
F1	Mild hydropic degeneration (25%)	Mild cell degeneration (20%)	0
F2	Mild hydropic degeneration (25%)	Mild cell degeneration (20%)	0
F3	Mild hydropic degeneration (20%)	Mild cell degeneration (15%)	0
Normal	0	0	0

Figure 3 exhibits the reading of the liver organs. The negative control group that did not receive any drugs suffered the most significant damage, with a rate of 55%. The F3 group experienced the lowest rate of organ damage (20%) among the treatment groups, followed by the F2 and F3 groups (25%) as well as the sappanwood, red ginger, and positive control groups (30%). Meanwhile, in the reading of the spleen organs, there was no organ damage.

DISCUSSION

Several studies have reported that sappanwood contains phytochemical constituents. Some of the phytochemical constituents are xanthone, coumarin, chalcone, flavone, homoisoflavonoid, brazilin, brazilein, campesterol, stigmasterol, and β-sitosterol (Nirmal et al. 2015, Bukke et al. 2015). Brazilin is the main phytochemical constituent in sappanwood. It has been reported to have pharmacological activities useful in treating rheumatoid arthritis, including anti-inflammatory, analgesic, antioxidant, and anticonvulsant activities (Jung et al. 2015, Kim et al. 2015).

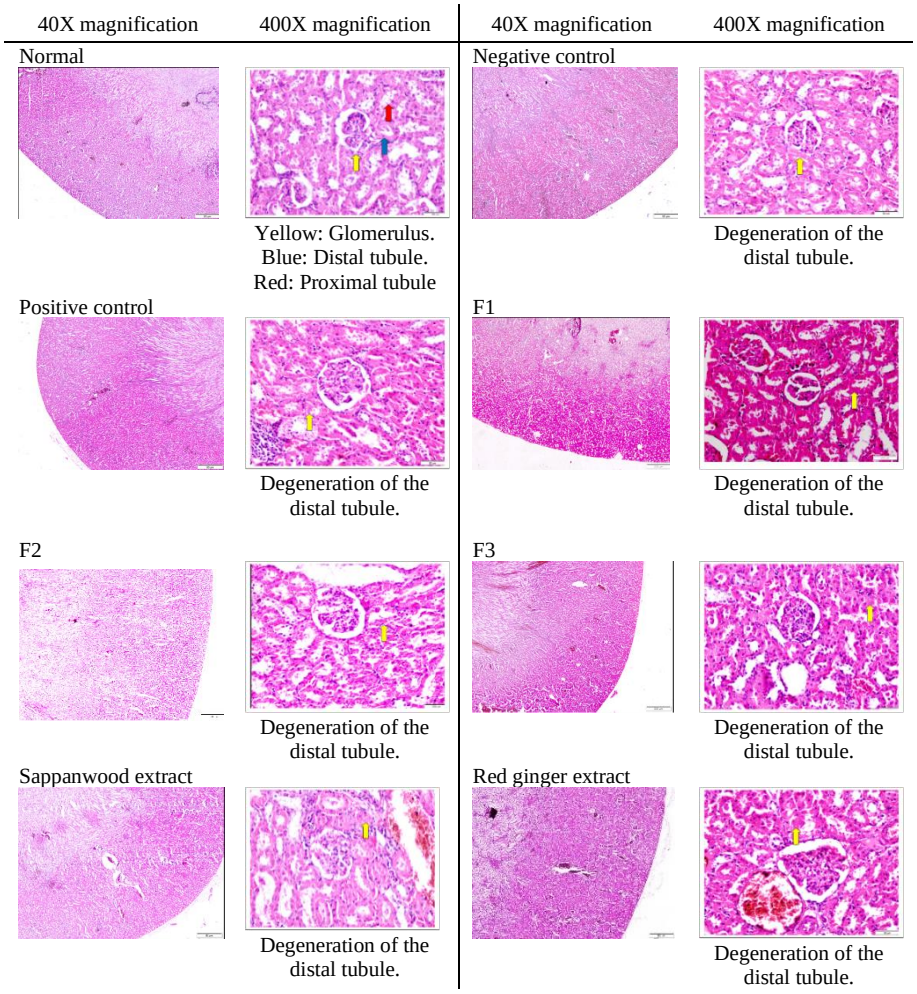

Figure 2. The results of microscopic observations on the kidney organs.

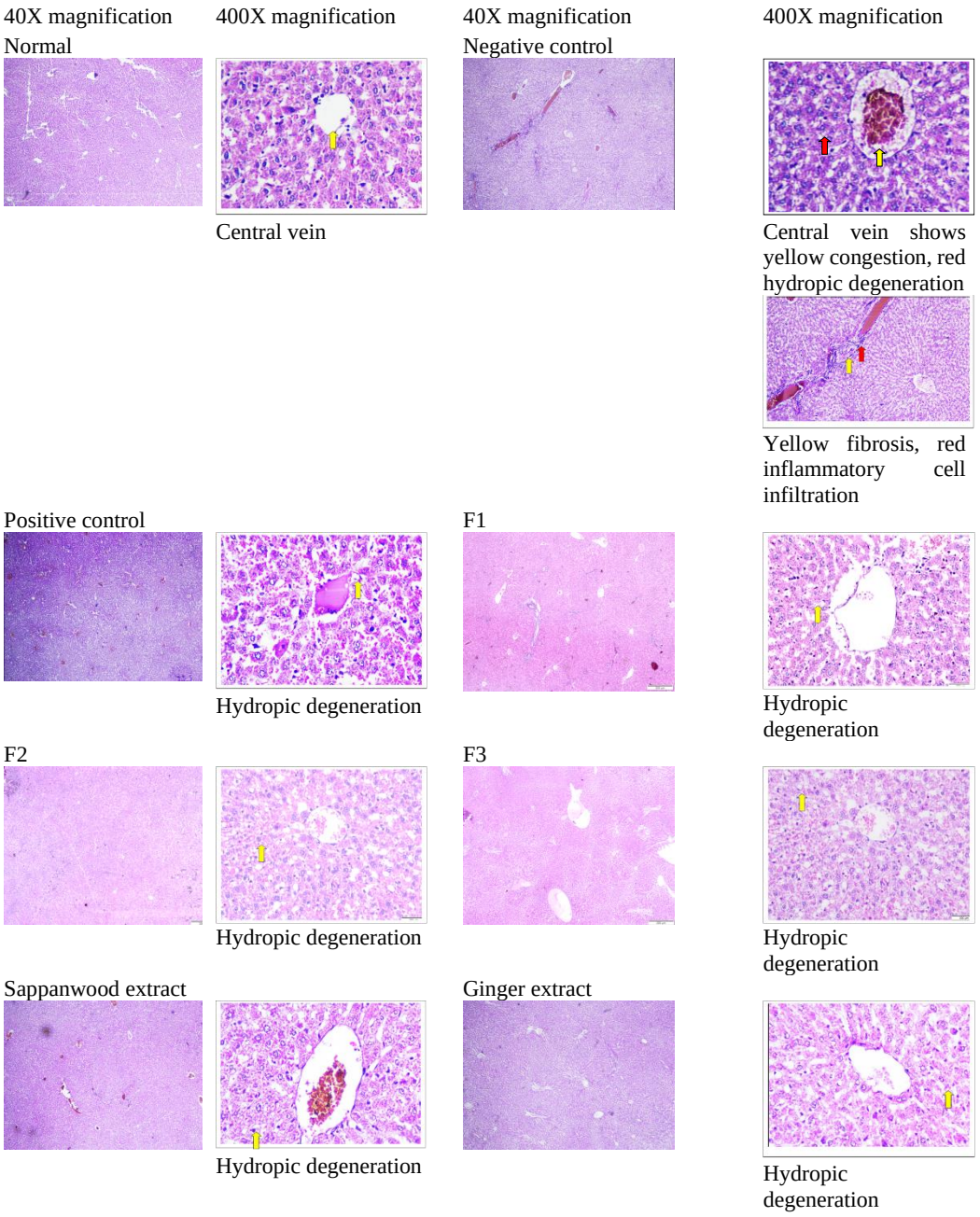

Figure 3. The results of microscopic observations on the liver organs.

Red ginger contains several bioactive compounds, i.e., 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, 8-shogaol, 10-shogaol, zingerone, 6-paradol, 6-dehydrogingerdione, 10-dehydrogingerdione, 6-gingerdione, 10-gingerdione, and 1-dehydro-6-gingerol. The pharmacological effects of red ginger include analgesic, anti-inflammatory, hepatoprotective, nephron protector, and antioxidant properties, which are useful in treating rheumatoid arthritis (Mbaveng & Kuete 2017, Ezzat et al. 2018).

Rattus norvegicus rats injected with complete Freund's adjuvant were used to evaluate the toxicity of the combination of sappanwood and red ginger extracts. Significant weight changes in the body and internal organs are considered a sensitive indicator of exposure to toxic substances. Due to their sensitivity in predicting toxicity and correlation with organ histopathological changes, the organ weight of the liver, kidney, and spleen can determine toxic effects. Increased liver, kidney, and spleen weight indicates organ hypertrophy, directly indicating chemical or biological toxicity (Amna et al. 2013, Ping et al. 2013). In this study, there was no organ hypertrophy in all test groups.

The toxic effects of xenobiotics can be seen in the liver and kidneys. The liver plays a role in drug

metabolism, transportation, and the clearance of foreign substances (Corsini & Bortolini 2013). The kidney is the main organ for drug secretion. While the spleen is essential in regulating the body's immunity, damage to the organ can cause immune disorders and splenomegaly (Bronte & Pittet 2013, Wang et al. 2019, Lees et al. 2020). In this study, the microscopic examination revealed no significant changes according to the observed parameters. Organ preparations observed under a light microscope in all fields of view did not indicate any adverse effects in the rats treated with extracts.

Damage to the kidney can be caused by toxic substances that enter the body. The primary function of the kidney is to excrete the remnants of the digestive system, which can be identified through histological structural changes, including cell degeneration and necrosis. Cell degeneration is an abnormality in cells that occurs due to light injury, which affects the structure of the cell. It causes disruptions to metabolic processes in the body. The destruction of proximal tubular epithelial cells is a symptom of cell necrosis, a form of cell death (Miller & Zachary 2017).

Viral infections can lead to the growth of benign or, more often, cancerous viruses that cause histological changes in the kidney. Due to the large number of compounds entering the tubules, the malignant effect may be heightened. This can cause the cells in the kidney to undergo necrosis. Various factors that can induce this condition include high levels of toxins such as phosphorus, poisonous mushrooms, and arsenic (Suhita et al. 2013).

Hepatic damage is indicated by two histological changes: hepatocytes and necrosis. The two types of damage in hepatocytes are hydropic degeneration and fatty degeneration. Hydropic degeneration is a condition in which cell damage is characterized by swelling of the cytoplasm, which is an excessive accumulation of fluid due to the inability of cells to maintain homeostasis (Berata et al. 2015). Fatty degeneration is the final stage of hydropic degeneration, which has suffered permanent (irreversible) damage. Fatty degeneration occurs when fat accumulates in the cytoplasm of liver cells (Sangi 2016). If the cell condition worsens over time, it can cause permanent damage. Furthermore, the cells may experience death or necrosis, which can cause symptoms such as nuclear chromatin. Nuclear changes that may happen include agglomeration (pyknosis), rupture (karyorrhexis), and dissolution (karyolysis) (Kumar et al. 2013).

Strength and limitations

This study evaluated the toxic effects caused by the combination of sappanwood and red ginger ethanol extracts for the treatment of rheumatoid arthritis in rat models. However, due to the limited dose of extracts in the combination formula, this study was unable to provide more representative findings for determining the optimal combination dose. Further research is required to determine the optimal dose for the safe treatment of rheumatoid arthritis in humans.

CONCLUSION

There was no fatality recorded during toxicity testing after the combination of sappanwood and red ginger ethanol extracts was administered to rats injected with complete Freund's adjuvant. The measurement of body weight and organ weight (i.e., the liver, kidney, and spleen) did not reveal any toxic effects. Additionally, the observed organs did not show any histopathological changes in rats treated with the extract.

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Conflict of interest

None.

Ethical consideration

This study obtained ethical clearance from the Health Research Ethics Committee, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, with registration No. 147/EC/KEPK/FKUA/2022 on 8/8/2022.

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Author contribution

NAS collected and analyzed the data and wrote the manuscript. T collected the data and wrote the manuscript. S collected and analyzed the data. FIS also collected and analyzed the data.

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Original Research Report

EFFECTS OF *Moringa oleifera* EXTRACT AS AN IMMUNOMODULATOR OF LYMPHOCYTE CELLS AND MACROPHAGES IN BALB/c MICE INFECTED WITH *Plasmodium berghei*

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ABSTRACT

Malaria is an infectious disease caused by protozoa of the genus *Plasmodium*. In Indonesia, this disease remains a health concern to be resolved. Due to its high prevalence in eastern Indonesia, it is challenging to eradicate this disease. As an herbal alternative for disease eradication, *Moringa oleifera* that contains various substances is believed to have an antimalarial activity. This study aimed to determine the effectiveness of *Moringa oleifera* leaf extract in increasing immune cells and eradicating parasites by using mice infected with *Plasmodium berghei*. The research was conducted in vivo on BALB/c strain mice (n=40) that were already infected with *Plasmodium berghei*. *Moringa oleifera* leaf extract at 25%, 50%, and 75% concentrations was administered orally every day to the mice, while a peripheral blood smear was performed to evaluate parasitemia levels and macrophage activation. A complete blood count was also performed after all tests on the mice were completed. Statistical analysis was performed using the one-way analysis of variance (ANOVA) test with α=0.05 and 95% confidence interval (CI). The results showed that the administration of *Moringa oleifera* leaf extract at 25%, 50%, and 75% concentrations caused varying degrees of parasitemia compared to the negative group ($p<0.05$). The group that received the extract at 50% concentration differed significantly from the control groups regarding the number of activated macrophages. The results of the complete blood count indicated immunomodulatory effects through the presence of diverse immune cell types. In conclusion, *Moringa oleifera* leaf extract suppresses *Plasmodium berghei* infection and enhances immune cell stimulation.

Keywords: Immunomodulator; macrophage; malaria; lymphocyte; *Moringa oleifera*

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Highlights:
1. This study assessed the immunomodulatory potential of *Moringa oleifera*, which may serve as a natural source for antimalarial treatment.
2. *Moringa oleifera* extract can act as an immunomodulator due to its suppressive effect on *Plasmodium berghei* infection.

INTRODUCTION

Indonesia is among the countries known for having a tropical climate. The tropical climate is like a double-edged knife, presenting both advantages and disadvantages. One of the benefits associated with a tropical environment is the consistent exposure to sunlight throughout the year. Unfortunately, it also entails disadvantages such as higher temperatures, the absence of the winter season, and certain health concerns. The health concerns arising from this climatic condition encompass the transmission of diseases spread by insects. *Anopheles* mosquitoes are a kind of insect that has a high degree of adaptability and can thrive in tropical conditions (Yahya et al. 2020). These mosquitoes are capable of sustaining their own lives while simultaneously spreading malaria. The disease is a result of a

Plasmodium sp. infection. Malaria exemplifies a pathological condition that targets the red blood cells. Consequently, complications may arise, particularly in relation to the quantity of red blood cells (Gultom et al. 2019).

The life cycle of malaria is typically categorized into three distinct stages: pre-erythrocytic, erythrocytic, and transmission. Upon the act of blood-feeding by a female Anopheles mosquito, the sporozoites are released into the host's bloodstream through the mosquito's saliva (Gultom et al. 2019). This particular stage is the start of the malaria life cycle. Initially, the sporozoites will penetrate the liver cells and form merozoites during the pre-erythrocytic stage. Merozoites will begin infecting erythrocytes and forming schizogony following the lysis of liver cells. This stage is known as the erythrocytic stage (Gultom et al. 2019). Upon the lysis of red blood cells, merozoites are released and subsequently proceed to re-infect these cells. After several cycles, gametocytes will develop and migrate to the mosquito's body when the mosquitoes feed on human blood. This process is referred to as the transmission stage.

The process of gametocyte fertilization results in the formation of a zygote, which subsequently develops into an oocyst and sporozoites. In addition to its detrimental effects on red blood cells and liver cells, malaria has the capacity to clog small blood vessels, including those found in the brain. This condition may induce tissue hypoxia (Fitriany & Sabiq 2018, Lee et al. 2022). The lethality of malaria is evident from its life cycle, particularly when inadequate treatments are applied.

In recent decades, malaria has emerged as a major problem in Indonesia. At the endemic level, malaria has had a rather benign impact on the community, despite the high prevalence of cases. According to the 2021 data provided by the Ministry of Health of the Republic of Indonesia, the number of active malaria cases was approximately 94,610 (Ministry of Health of the Republic of Indonesia 2022). The provinces of Papua, West Papua, and East Nusa Tenggara had the highest distribution of reported cases. Numerous attempts have been made by researchers to address this issue. Wardani et al. (2020) explored the potential of using *ashitaba* (*Angelica keiskei* (Miq.) Koidz.) stem ethanol extract as an herbal medicine for malaria. However, the current prevalence of malaria cases remains high, indicating that prior research efforts have not effectively contributed to the eradication of malaria. Additionally, there is a concerning emergence of malaria drug resistance, necessitating the development of novel antimalarial medications (Ministry of Health of the Republic of Indonesia 2022).

Moringa oleifera, commonly known as the miracle plant, possesses the potential to treat numerous health problems. *Moringa oleifera* harbors a range of secondary metabolites, including saponins, tannins, terpenoids, vitamin C, and flavonoids. The flavonoid compounds may exist in various forms, such as chalcones, kaempferol, alkaloids, phenols, and quercetin (Rivai 2020). The flavonoid content present in Moringa leaves is abundant in antioxidants. The antimalarial effect of the antioxidants has been reported to occur through the inhibition of malaria pathogen proliferation and the enhancement of immune cells (Veronica et al. 2020). One of the notable benefits of *Moringa oleifera* is its widespread availability in both local markets and supermarkets. On the other hand, the primary emphasis of dihydroartemisinin-piperaquine (DHP) as a gold standard therapeutic intervention is on its antimalarial activities rather than its role as an immunomodulator. Immuno-modulatory agents have the capacity to increase immune cells, including T lymphocytes and other activated cells, thereby accelerating the healing process when combating pathogens (Veronica et al. 2020). Therefore, the purpose of this study was to examine the effect of *Moringa oleifera* leaf extract on parasitemia and immune cells, specifically macrophages and lymphocytes.

MATERIALS AND METHODS

This was a quasi-experimental study conducted in the animal breeding and research laboratory of the Faculty of Medicine and Health Sciences, Universitas Warmadewa, Denpasar, Indonesia. The experimental samples were BALB/c strain mice, around 7 weeks old and weighing approximately 25 grams. According to Federer's formula, a total of 40 mice were divided into five groups, with each group consisting of eight mice (Arwati et al. 2021, Molek et al. 2023).

The first group (negative control) received only water. The second, third, and fourth groups received *Moringa oleifera* leaf extract at varying concentrations of 25%, 50%, and 75%, respectively. The fifth group (positive control) received dihydroartemisinin-piperaquine at a dose of 187.2 mg/kg BW (Arwati et al. 2021). The dihydro-artemisin-piperaquine was manufactured in Indonesia by PT Mersifarma TM (6O96'15.8"S 106O78'73.9"E), with the batch number MTA-124486296. The *Moringa oleifera* leaves were acquired from a local market in Denpasar (08°14′17″S 115°05′02″E) and identified according to their morphology.

Moringa oleifera is a shrub or tree with strong roots. The plant is characterized by its long lifespan, brittle

woody stems, upright growth, dirty white hue, thin skin, rough surface texture, and rare branching (Benyamin 2023). The morphology of *Moringa oleifera* leaves is characterized by an oval shape and blunt edges. The size of the leaves is rather small. A single stalk of *Moringa oleifera* consists of a compound of leaves, as shown in Figure 1. *Moringa oleifera* has yellowish-white flowers that bloom all year with a distinctive fragrance. *Moringa oleifera* also bears long and triangular fruits with a length of about 20–60 cm. The fruits are typically green throughout their early stages of growth, and they turn brown as they ripen (Pratiwi 2018).

Figure 1. Morphological appearance of the *Moringa oleifera* stalk.

The preparation of *Moringa oleifera* leaf extract was performed by soaking refined dried leaves in 70% alcohol for three days. Afterward, the substance was filtered and evaporated to obtain a concentrated extract (Azzahra & Hayati 2019). The *Moringa oleifera* leaf extract was then diluted using distilled water in the appropriate ratios to achieve the desired extract concentrations of 25%, 50%, and 75%. Various concentrations of *Moringa oleifera* leaf extract were utilized as independent variables in this study. Prior to the experiment, all mice underwent a seven-day acclimatization. After the acclimatization period, blood containing 0.2 mL of the *Plasmodium berghei* parasite was injected into the peritoneal cavity of the mice (Farizal et al. 2020). The intraperitoneal injection was administered at an angle of 100 degrees relative to the abdomen, with a small inclination towards the midline. The procedure was performed with a cautious approach and at a moderate elevation to prevent any injury to the bladder or liver (Samson et al. 2019, Refdanita et al. 2020). The independent variables were administered orally to the mice starting on day 0 (two hours after infection) until day 4. The dosage protocol included the administration of 0.5 mL of *Moringa oleifera* leaf extract per 25 g of body weight in mice, with a frequency of once daily or a 24-hour time interval (Mustofa et al. 2019, Arwati et al. 2021). On day 5, only observation was conducted, with no further administration of the independent variables. The observation continued until day 6, which marked the seventh day of infection (Mustofa et al. 2019).

The results of administering the independent variables were observed on a daily basis during the experiment from day 1 to day 6. Consequently, six data sets were obtained for each independent variable (Kweyamba et al. 2019). Blood samples were taken from the tails of the experimental mice every day prior to treatment from day 1 to day 4, as well as 24 hours after treatment for days 5 and 6. The macrophage count was examined using peripheral blood smears and observed under a microscope following the Giemsa staining. In addition, a complete blood count was carried out to analyze the lymphocyte cells (Taek 2018, Hermanto et al. 2022). The macrophage morphology was observed in five fields of view. The number of infected erythrocytes was determined by assessing the degree of parasitemia across five fields of view and dividing by 1,000. This analysis showed whether the results represented normal erythrocytes in the five fields of view (Mustofa et al. 2019, Arwati et al. 2021, Juniantara et al. 2022).

All data obtained were analyzed using a one-way analysis of variance (ANOVA) test with a 95% confidence interval (CI) and $p<0.05$. The statistical analysis was conducted using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, N.Y., USA) (Mishra et al. 2019). Ethical approval for this research was obtained from the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Warmadewa, Denpasar, Indonesia, with registration No. 320/Unwar/FKIK /EC-KEPK/IV/2023 dated 3/4/2023.

RESULTS

Degree of parasitemia in all groups

During the experiment in this study, it was observed that each group had various degrees of parasitemia with different patterns of fluctuations (Table 1). The mean degree of parasitemia in Group 1, which served as the negative control, was determined to be 14.68% on day 1. There was an increase seen on day 2, with a recorded mean degree of 16.67%. This trend continued until the last day, when it reached around 34.60%.

In this study, Group 2 received *Moringa oleifera* leaf extract at a concentration of 25%. On day 1, the mean degree of parasitemia was recorded as 7.77%, which subsequently climbed to 15.7% on day 2. However, on day 6, this group exhibited a mean degree of parasitemia of 10.2%, indicating a decrease compared to the previous day.

In the experiment involving Group 3, *Moringa oleifera* leaf extract was administered at a 50% concentration. On day 1, the mean degree of

parasitemia observed in this group was 7.34%, which subsequently increased to 13% on day 2. Nevertheless, on day 6, the group showed a reduction in the degree of parasitemia, which decreased to 7.29%.

Group 4 received *Moringa oleifera* leaf extract at a 75% concentration. The degree of parasitemia in this group exhibited a reduction from 8.6% on day 1 to 7.16% on day 2. The observed group exhibited an escalation in the degree of parasitemia until they recorded a decline to 9.6% on day 6.

Group 5, which served as the positive control, received dihydroartemisinin-piperaquine at a dose of 187.2 mg/kg BW. The group exhibited a progressive decline in the degree of parasitemia. The mean value of the degree on day 1 was recorded as 6%, and it continued to decrease to 2% on day 6.

The results of the one-way ANOVA test indicated that the administration of Moringa leaf extract to mice infected with *Plasmodium berghei* led to a statistically significant reduction in the degree of parasitemia ($p<0.05$). Differences in the degree of parasitemia were seen between the positive control group and the groups given *Moringa oleifera* leaf extract at concentrations of 50% and 75%. However, the observed differences were statistically significant only until day 4.

Table 1. Degree of parasitemia observed in each group on a daily basis.

Day	Mean (%) degree of parasitemia					
	Group 1	Group 2	Group 3	Group 4	Group 5	p
1	14.68	7.77	7.34	8.60	6.23	0.00*
2	16.76	15.70	13.00	7.16	8.00	0.00*
3	22.73	15.30	13.01	13.16	6.55	0.00*
4	24.30	14.40	11.34	11.90	6.00	0.00*
5	25.73	10.40	8.61	11.23	4.30	0.00*
6	34.60	10.20	7.29	9.60	3.75	0.00*

Note: Significance level at $p<0.05$*.

Activated macrophages in all groups

The observation revealed that the process of macrophage activation had occurred (Table 2). In Group 1, activated macrophages were obtained with a mean value of 2.83 on day 1. On day 2, there was a decline in the number, resulting in a mean value of 1.80. Subsequently, it reached a mean value of 2.2 on the final day.

Group 2 exhibited the activation of macrophages on day 1, with an observed mean value of 3.86. The value showed a rise to 4.5 on day 2. On the final day of the experiment, the group demonstrated a statistically significant rise in the mean value of activated macrophages.

On day 1, it was found that the mean activated macrophages in Group 3 were 6.13. On day 2, the measurement of activated macrophages revealed a reduction in the mean value to 6. On the last day of the experiment, there was a decline in the mean value of activated macrophages to 5.

The macrophages that were activated in Group 4 had an approximate value of 7.38 on day 1. The value subsequently declined to 4.29 on day 4. The activated macrophages in Group 4 exhibited a gradual decrease until day 4, followed by an upward trend that peaked at a mean value of 6.43 on the final day of the experiment.

In comparison to the treatment groups, it was observed that Group 5 had a mean activated macrophage value of 5 on day 1. The observed changes in the activated macrophages of this group were rather consistent. On day 2, there was a rise in the mean value to 6.33. On the final day of the experiment, the mean value of activated macrophages reached 6.5. According to the results of the one-way ANOVA test, the administration of *Moringa oleifera* leaf extract to mice that were infected with malaria resulted in a statistically significant increase in the number of activated macrophages observed from day 1 to day 4.

Table 2. Activated macrophages observed in each group on a daily basis.

Day	Mean** activated macrophages					
	Group 1	Group 2	Group 3	Group 4	Group 5	p
1	2.83	3.86	6.13	7.38	5.00	0.00*
2	1.80	4.50	6.00	4.29	6.33	0.00*
3	2.00	5.00	4.29	3.88	5.00	0.00*
4	2.50	3.33	5.13	2.75	4.33	0.01*
5	2.00	4.50	5.00	4.14	5.67	0.15
6	2.20	5.00	5.00	6.43	6.50	0.17

Notes: Significance level at $p<0.05$*; total macrophages in five fields of view**.

Complete blood count in all groups

According to the complete blood count of all groups, there was no statistically significant difference in lymphocyte cells ($p=0.63$) (Table 3). Nevertheless, when considering the average value, Group 3 exhibited the highest average. In the analysis of basophil cells, a statistically significant increase ($p=0.02$) was observed, with Group 3 having the highest number. Furthermore, the presence of healthy red blood cells suggested that the blood cells were not affected by *Plasmodium sp.* infection ($p=0.04$). Among the groups studied, Group 5 achieved the highest score, followed by Group 4 and Group 3.

Table 3. Results of the complete blood count.

Immune cells	Complete blood count (cell/mm^3)					
	G1	G2	G3	G4	G5	p
Neutrophil	1.30	2.94	0.18	0.33	0.38	0.13
Lymphocyte	3.24	3.84	5.66	4.56	2.15	0.63
Eosinophil	0.11	0.03	0.04	0.03	0.00	0.43
Basophil	0.86	2.56	0.56	0.93	2.15	0.02*
Erythrocyte	5.54	6.77	6.95	7.56	7.86	0.04*

Note: Significance level at p<0.05*. G=Group.

DISCUSSION

Degree of parasitemia in all groups

This study revealed significant improvements throughout all observation days in the treatment groups administered with *Moringa oleifera* leaf extract. This might be due to the presence of many secondary metabolites within *Moringa oleifera* leaves (Rivai 2020, Islam et al. 2021). Flavonoids in *Moringa oleifera* leaves act as antioxidants, which can inhibit the growth of malaria pathogens and stimulate immune cells. A new permeation pathway can inhibit the process of membrane formation on Plasmodium, thereby preventing the degradation of hemoglobin (Marhaeni 2021). Consequently, *Plasmodium sp.* will be deprived of a vital nutrient source, leading to an inability to develop further. This is also important to prevent anemia, which usually occurs in malaria cases. Anemia has a tendency to progress into more severe illnesses, such as blackwater fever (Veronica et al. 2020).

Kaempferol in *Moringa oleifera* leaves has been found to have significant inhibitory effects on the production of reactive oxidative stress (ROS) and the process of fat peroxidation. These processes are known to contribute to the damage of cell membranes in malaria cases. Furthermore, *Moringa oleifera* leaves contain vitamin C, a compound that can enhance the enzymatic breakdown of hemoglobin (Hb) into ferrous iron (Fe2+). It is crucial that this action occur before malaria parasites start the degradation of infected hemoglobin. If the infected hemoglobin is damaged, it will result in the generation of ferric iron (Fe3+) and stimulate the release of electrons. Consequently, reactive oxidative intermediates (ROI) will be formed, leading to the production of hydrogen peroxide (H2O2) and damage to the host body. Vitamin C can also slow down the process of hemolysis and combat toxic cells as it possesses antioxidant properties (Veronica et al. 2020).

There was a statistically significant difference (p<0.05) in the degree of parasitemia between the negative control group and all other groups throughout all observation days. The findings of this study indicated that *Moringa oleifera* leaf extract had antimalarial activity. In contrast to the positive control group, the administration of the extract at a 25% concentration showed a significant effect after day 2. The administration of the extract revealed significance starting from day 2 until day 4 at 50% concentration and from day 1 through day 6 at 75% concentration. The decrease in parasitemia observed in the treatment groups could not resemble the pattern in the positive control group, despite the presence of antimalarial activity. The findings suggest that the efficacy of *Moringa oleifera* leaf extract as an antimalarial medication falls short in comparison to dihydroartemisinin-piperaquine. However, it is important to emphasize that the treatment group receiving *Moringa oleifera* leaf extract at 50% concentration showed a similar effect on the degree of parasitemia when compared to the positive control group. The provided evidence can serve as a starting point for future research projects (Mustofa et al. 2019, Obediah & Christian Obi 2020).

Activated macrophage in all groups

The difference in the activated macrophages was only significant until day 4, particularly in the group receiving *Moringa oleifera* leaf extract at 50% concentration compared to the negative control group. *Moringa oleifera* is abundant in components that can stimulate interleukin-2 (IL-2) activity. As IL-2 stimulates lymphocyte cell proliferation, this substance is crucial after an antigen-presenting cell has presented *Plasmodium sp.* antigen to lymphocyte cells. After proliferating, lymphocyte cells will differentiate into T helper 1 (Th1) cells, which will activate macrophages after interferon gamma (IFN-γ) production (Widiani et al. 2021). Additionally, interferon gamma stimulates the transformation of monocytes into macrophages. As innate immune cells, macrophages will respond first to phagocytose pathogens (Mustofa et al. 2019, Subryana et al. 2020).

Complete blood count in all groups

An immunomodulator refers to a compound that possesses the ability to improve the performance of the immune system. The immune system consists of a variety of cells, including neutrophils, basophils, eosinophils, and lymphocytes (Perdana 2021). Lymphocyte cells are primarily involved in the immune response against pathogenic micro-organisms, the elimination of tumor cells or malignancies, and the prevention of organ transplant rejection. There are various factors that can contribute to an increase in lymphocyte cell count. Lymphocyte cells have several types, such as T helper lymphocyte cells, cytotoxic T lymphocyte cells, and B lymphocyte cells (Amran & Al Qarni 2019, Erniati & Ezraneti 2020).

T helper cells play a more significant role in activating other immune cells, including neutrophils, eosinophils, basophils, and B lymphocyte cells. By increasing basophils, T helper 2 (Th2) and T helper 9 (Th9) cells specifically combat parasitic infections such as malaria. Cytotoxic T cells destroy cells infected by pathogens. B lymphocyte cells produce antibodies that combat pathogens. In essence, lymphocyte cells have a broad spectrum of functions, so the role of lymphocyte cells in the immune response to malaria infection could not be specified in this study. Due to the inability of a complete blood count to determine the specific type of T helper, T cytotoxic, and B lymphocyte cells that had increased, additional research is required to analyze the count of lymphocyte cells according to their respective types (Prakoeswa 2020).

In this study, a significant increase in basophils was observed in the complete blood count. Basophils are cells that eliminate parasites after receiving information, specifically when interleukin-4 (IL-4) is produced by Th2 cells. Thus, an increase in basophils indirectly indicates an increase in Th2 cells (Tania 2020, Widiani et al. 2021). However, the complete blood count in this study also needs improvement. During the transport to the laboratory, the blood samples were placed in a cooler box, which caused several blood clots to form. Numerous cells were likely lost, affecting the results and causing discrepancies. There is a minimum quantity of blood that must be examined to perform a complete blood count, which makes it difficult to examine the blood of mice. Approximately 7.7 mL/kg BW was the maximum quantity of blood that could be extracted from mice. Since the mice in this study weighed between 25 and 30 grams, only about 1.9 mL of blood could be extracted before the mice died (Fatmawati et al. 2018).

This study did not assess potential adverse effects or toxicity. It is suggested that toxicity testing may be conducted in future research. The use of animal models in this study poses uncertainty regarding the generalizability of the observed effects to humans. However, human trials will proceed following the conclusion of this animal testing. In addition, *Plasmodium berghei*, a malaria species that infects rodents, is internationally recognized for its utility in studies of malaria remedies for humans. This is mostly due to its shared pathogenesis process with *Plasmodium falciparum*, which infects humans (Intan et al. 2020).

Strength and limitations

The study provides information regarding the effects of *Moringa olefera* leaf extract on both antimalarial activity and immunomodulatory properties. Furthermore, this study can serve as a valuable resource for future research aiming to explore the potential of *Moringa oleifera* leaf extract as a therapeutic intervention for malaria and immunomodulation. However, it is important to acknowledge the limitations of this study, particularly in relation to the samples used for the complete blood count analysis. Several issues occurred during the study, including blood clots and frozen blood samples. Consequently, only a limited volume of blood samples was eligible to be analyzed. The findings from the complete blood count indicated the absence of cluster of differentiation 4 (CD4) among the total lymphocytes, which contributed to the severity of the malaria infection.

CONCLUSION

Moringa oleifera leaf extract has antimalarial activity and immunomodulatory effects. However, it is not as effective as dihydroartemisinin-piperaquine, which serves as the established standard medicine for malaria. Further research is required to explore varying intervals of dosage administration. The effectiveness of a complete blood count to assess lymphocyte count is limited due to the numerous lymphocyte types. Therefore, it is necessary to use a more precise method for assessing lymphocytes in future studies. It is also crucial to take the storing method of blood samples into account to preserve the quality of the samples.

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Conflict of interest

None.

Ethical consideration

This study obtained ethical approval for research feasibility from the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Warmadewa, Denpasar, Indonesia, with registration No. 320/Unwar/FKIK /EC-KEPK/IV/2023 on 3/4/2023.

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Author contribution

PKDJ contributed to the conceptualization, study design, data collection, data analysis, data interpretation, methodology, manuscript writing, project administration, and content revision. PIBA contributed to the conceptualization, study design, data collection, data analysis, data interpretation, methodology, manuscript writing, content revision, supervision, and final approval. PMACPND contributed to the conceptualization, study design, data collection, methodology, and manuscript writing. DAASL contributed to the conceptualization, study design, content revision, supervision, and provision of samples. IKCAWS contributed technical and logistical support.

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Original Research Report

BIOFILM FORMATION IN *Staphylococcus aureus* AND COAGULASE-NEGATIVE *Staphylococcus*

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ABSTRACT

Staphylococcus spp. is typically a commensal microorganism that can exist in the human body without causing illness. However, such a bacterium has virulence factors, e.g., biofilm formation, which are important to note. Because biofilms shield bacteria from opsonophagocytosis and antimicrobial agents, they can cause persistent or chronic infections. Once they form biofilms, both *Staphylococcus aureus* and coagulase-negative *Staphylococcus* (CoNS) can potentially cause incurable infections. This study aimed to compare biofilm formation in *Staphylococcus aureus* and coagulase-negative *Staphylococcus* as a guide for the prevention and management of infection, thus maintaining and improving public health. This current study was analytic research with a cross-sectional design. It began by collecting the samples, identifying the species, and testing the biofilm production with a microtiter plate, which was then analyzed with an enzyme-linked immunosorbent assay (ELISA). Data analysis was conducted using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA). Comparison tests were conducted using an independent t-test. A value of $p<0.05$ was used as the cut-off to indicate significance. The total samples were 36 clinical isolates, consisting of 18 *Staphylococcus aureus* and 18 coagulase-negative *Staphylococcus*. The specimens consisted of 20 blood samples (55.6%) and 7 wound swabs (19.4%). The biofilm test on the samples showed that 83.3% of the samples produced biofilms. The data revealed that the isolates formed biofilms, with 14 isolates (38.9%) in the strong category, 10 isolates (27.8%) in the moderate category, and 6 isolates (16.7%) in each of the weak and non-existent categories. Both *Staphylococcus spp.* appeared to have a biofilm-forming activity, but coagulase-negative *Staphylococcus* appeared to be significantly more dominant ($p=0.008$) than *Staphylococus aureus*. Strong biofilm was produced by 61.1% of coagulase-negative *Staphylococcus* isolates. In conclusion, coagulase-negative *Staphylococcus* formed a stronger biofilm than *Staphylococcus aureus*. Its presence as an infection-causing bacteria, particularly in immunocompromised patients, should not be underestimated.

Keywords: Biofilm; *Staphylococcus*; human and health; immunocompromised patients

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Highlights:
1. The significance of *Staphylococcus aureus* and coagulase-negative *Staphylococcus*, which are more likely to infect immunocompromised patients, needed to be researched in greater depth.
2. Coagulase-negative *Staphylococcus* was found to form significantly more biofilm than *Staphylococcus aureus*.
3. Wound care and changing medical devices in immunocompromised patients on a regular basis may provide benefits to prevent biofilm formation by *Staphylococcus spp.*

INTRODUCTION

Staphylococcus is a genus of Gram-positive bacteria that commonly inhabits the skin and mucous membranes of humans and animals. Gram-positive cocci are also common isolates in the microbiology laboratory (Mahon & Lehman 2022). *Staphylococcus* can be classified into coagulase-

positive and coagulase-negative species based on their ability to produce the enzyme coagulase. The enzyme coagulase produced by coagulase-positive *Staphylococcus* causes blood to clot. The most clinically relevant coagulase-positive species is *Staphylococcus aureus* (*S. aureus*). This strain of bacteria is a major human pathogen that can cause a wide range of infections, including skin and soft tissue infections, pneumonia, endocarditis, and sepsis (Riedel et al. 2019, Mahon & Lehman 2022). *Staphylococcus aureus* inhabits several areas of human body and has the ability to cause infections in humans under certain conditions. Approximately 20–30% of the human population has *Staphylococcus aureus* colonization in the nose, throat, folds, and gastrointestinal tract (Tong et al. 2015).

Coagulase-negative *Staphylococcus* (CoNS), on the other hand, does not produce coagulase. Some of the clinically important coagulase-negative species include *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, and *Staphylococcus haemolyticus*. These species are commonly found on the skin and mucous membranes and are typically considered to be less virulent than *Staphylococcus aureus*. However, they can still cause a range of infections, particularly in individuals with compromised immune systems or who have indwelling medical devices (e.g., catheters or prosthetic devices), due to their ability to colonize (Águila-Arcos et al. 2017, Zheng et al. 2018). It is worth noting that coagulase-negative staphylococci are a common cause of nosocomial (hospital-acquired) infections. In many cases, these bacteria demonstrate resistance to multiple antibiotics (Águila-Arcos et al. 2017).

Biofilms are defined as a community of microbial cells permanently attached to each other on a surface (either inanimate or living organisms) by a matrix of extracellular polymeric substances (EPSs). The term biofilm was coined by John William Costerton in 1978 (Sagar et al. 2016). Biofilms are formed because microorganisms tend to aggregate and create a safe and comfortable environment for their community. As a survival mechanism, birofilms can lead to severe and chronic infections (Rasamiravaka et al. 2015). Biofilms on medical implants are one of the most troubling issues for medical practitioners due to the significant ability of bacteria to evade the body's immune system and antimicrobial agents, or antibiotics. Biofilms, which are an important virulence factor of *Staphylococcus spp.*, have an impact on the outcome of patients infected with *Staphylococcus spp.* (Nourbakhsh & Namvar 2016). Infectious conditions caused by biofilms that are not properly treated will result in antibiotic overuse, which will lead to resistance and increased mortality and morbidity due to unresolved sepsis.

The presence of coagulase-negative *Staphylococcus* does not receive the appropriate concern for its potential impact since *Staphylococcus aureus* infection is generally of more concern to clinicians. Unquestionably, the virulence factors of the two pathogens are distinct, but the process of treating an infection becomes equally challenging when biofilm is present (Águila-Arcos et al. 2017, Riedel et al. 2019). This study aimed to compare the biofilm formation of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* as a guide for the prevention and treatment of infections, as well as for the maintenance and improvement of good health in the community. It is anticipated that this study will encourage clinicians to be more vigilant, allowing the process of infection management to be more effective for both patients and doctors. In addition, unnecessary antibiotic use should be avoided so that resistance does not develop further.

MATERIALS AND METHODS

This was an analytical study with a cross-sectional design to compare biofilm formation in *Staphylococcus aureus* and coagulase-negative *Staphylococcus* (Kesmodel 2018). The study was conducted from July 2022 to March 2023. A total of 36 samples were obtained from patients with staphylococcal infections at Sanjiwani Gianyar Regional General Hospital, Gianyar, Indonesia. The research process continued at the Faculty of Medicine and Health Sciences, Universitas Warmadewa, Denpasar, Indonesia. Patients who participated in this study were those who had a *Staphylococcus spp.* infection or colonization and were hospitalized between October 2022 and January 2023.

The research process began with the identification of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* by culturing the samples on blood agar (BA) media. It was then followed by an incubation process for 24 hours at 37°C (Mahon & Lehman 2022). *Staphylococcus* isolates with positive catalase test results underwent further identification using a VITEK® 2 machine (BioMérieux, USA). This process was carried out in the microbiology laboratory of Sanjiwani Gianyar Regional General Hospital. Methicillin resistance was also determined using the disk diffusion method with a cefoxitin disc according to the Clinical and Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute 2022).

The biofilm assay was performed in several stages using a microtiter plate and a crystal violet staining assay in the microbiology laboratory of the Faculty of Medicine and Health Sciences, Universitas Warmadewa. The bacteria from the culture were

inoculated in 1% liquid glucose and 3 mL of phosphate-buffered saline (PBS) at pH 7 (Torlak et al. 2017, Omidi et al. 2020). The turbidity was measured with DENSICHEK® (BioMérieux, USA), and the range for a McFarland standard was 0.50–0.63. A total of 200 µL of suspension was transferred to a microtiter plate and incubated at 37°C for 48 hours without shaking. In each well of the microtiter plate, the remaining solution was discarded and rinsed with distilled water. This process was repeated in every step. Biofilm attached to the wells was stained with 200 µL of 0.1% crystal violet for 5 minutes. The microtiter plate was then rinsed, and 200 µL of 30% acetic acid was added to each well and allowed to dissolve for 5–15 minutes. The fluorescence intensity of crystal violet was measured with a microplate in an enzyme-linked immunosorbent assay (ELISA) reader using a wavelength of 620–670 nm. The methods used in this study referred to previous research with some modifications for method optimization (Samadi et al. 2017).

The collected data were then analyzed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) in two stages. The first step was a descriptive statistical analysis, which was conducted to describe the characteristics of each variable in this study. Data variables were expressed in the form of relative frequency (number and percent). The second stage was a bivariate analysis to determine whether there were significant differences in the ability to form biofilms on both types of *Staphylococcus aureus* and coagulase-negative *Staphylococcus*. The optical density cutoff (ODc) value was determined according to the quantitative value of biofilm. This was defined as the mean OD of the negative control +3 × standard deviation (SD) of the negative control. Biofilm formation by isolates was analyzed and categorized based on the absorbance of attached cells by crystal violet staining. The biofilm formation ability was then divided into 4 categories, i.e., none (OD≤ODc), weak (ODc<OD≤2xODc), moderate (2xODc<OD ≤4xODc), and strong (4xODc<OD) (Pompilio et al. 2020, Kasperski et al. 2023). A comparison test was performed using an independent t-test. The cutoff for statistical significance was $p<0.05$, and the precision value was determined by a 95% confidence interval (CI) (Banerjee 2014).

This study had received ethical approval from the Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Warmadewa, Denpasar, Indonesia, with registration No. 305/ Unwar/FKIK/EC-KEPK/II/2023 on 23/2/2023.

RESULTS

A total of 36 samples were obtained, comprising 18 isolates of *Staphylococcus aureus* and 18 isolates of coagulase-negative *Staphylococcus*. Thirteen samples (36.1%) were collected from patients in the age range of 46–65 years. The total sample showed a balanced representation of both genders. The largest specimen type utilized in this study was blood, accounting for a total of 20 samples (55.6%). This was followed by 7 wound swabs (19.4%) and 5 sputum samples (13.9%). The prevalent diagnoses seen among the patient population consisted of pneumonia (12 cases, 33.3%), skin and soft tissue infection (8 cases, 22.2%), and chronic kidney disease (CKD) (6 cases, 16.7%). The complete results are visually presented in Figures 1 and 2. Out of the total number of samples tested for sensitivity to cefoxitin, 19 samples (52.8%) exhibited positive results on the cefoxitin test. These positive results were indicative of the presence of meticillin-sensitive *Staphylococcus aureus* (MSSA) and meticillin-resistant *Staphylococcus aureus* (MRSA).

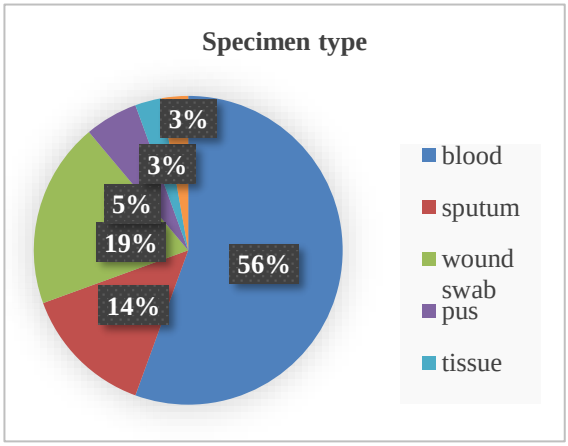

Figure 1. The distribution of specimen types, expressed in percentage (n=36).

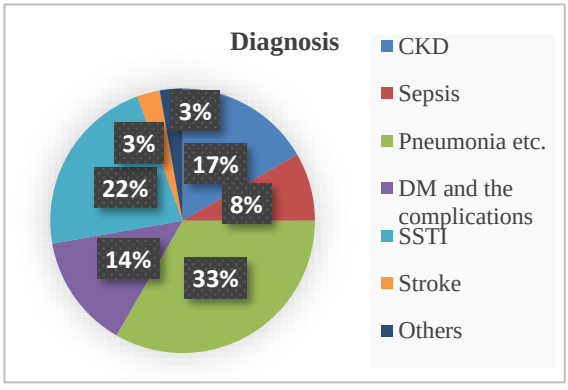

Figure 2. The distribution of diagnoses among the isolates, expressed in percentage (n=36).

The results of the biofilm assay conducted on the samples indicated that 83.3% of the 36 isolates exhibited biofilm formation following incubation for 48 hours at a temperature of 37°C in a 1% glucose solution. The mean optical density of the biofilm produced was determined to be 0.427 using an ELISA reader. The lowest value observed was 0.02, while the highest value recorded was 1.055.

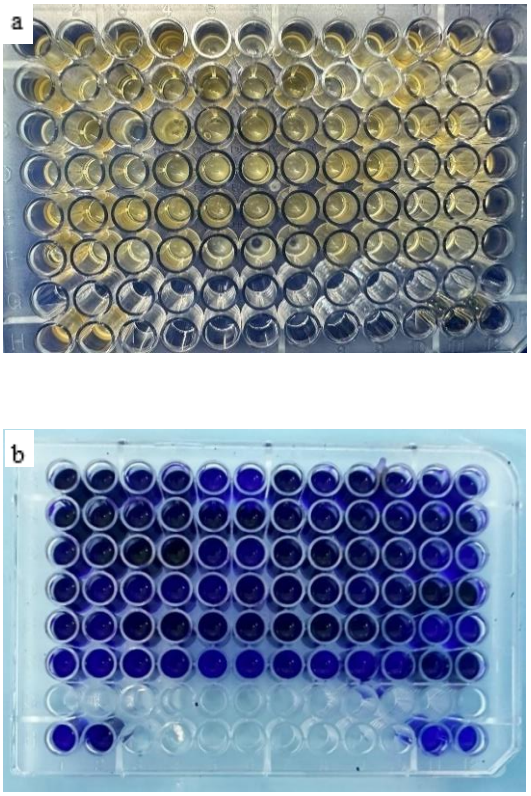

Figure 3. Biofilms appeared as white layers on the bottom of a microtiter plate, both before (a) and after (b) staining with crystal violet.

The data relating to the ability of biofilm formation indicated that a majority of the isolates exhibited strong biofilm formation with 14 isolates (38.9%). This was followed by 10 isolates (27.8%) displaying moderate biofilm formation. Additionally, 6 isolates (16.7%) exhibited weak biofilm formation, while an equal number of isolates showed no biofilm formation. The mean optical density of *Staphylococcus aureus* isolates was found to be 0.277, while the average optical density of coagulase-negative staphylococci isolates was determined to be 0.579. Table 1 presents more details on the classification of biofilms generated by the two distinct groups of *Staphylococcus*.

Table 1. Comparison of the strength of biofilms that were produced.

		Staphylococcus spp.	
		S. aureus	CoNS
Biofilm	Strong	3 (16.7%)	11 (61.11%)
	Moderate	3 (16.7%)	7 (38.8%)
	Weak	6 (33%)	0
	None	6 (33%)	0
Total		18 (100%)	18 (100%)

Note: CoNS = Coagulase-negative *Staphylococcus*.

The ability for biofilm formation was observed in both types of *Staphylococci*. However, it was significantly more evident in coagulase-negative *Staphylococcus* (p=0.008).

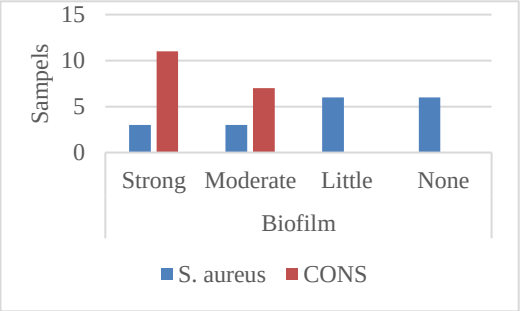

Figure 4. Overview of the ability of biofilm formation.

DISCUSSION

Both *Staphylococcus aureus* and coagulase-negative *Staphylococcus* strains are known for their ability to form biofilms, which are one of the virulence factors. This feature contributes to treatment difficulties because these biofilms are resistant to both the immune system and antibiotics. This resistance is especially prevalent among patients who use medical devices such as infusion catheters, central venous catheters, implanted devices (e.g., pacemakers), and urinary catheters (Silva-Santana et al. 2016, de Oliveira et al. 2021). Furthermore, the formation of biofilm on inadequately cleansed wound surfaces impedes wound healing.

The most common specimen type observed in this study was blood, followed by wound swabs and sputum. Meanwhile, the most prevalent diagnoses were pneumonia, skin and soft tissue infections, and CKD. Patients diagnosed with CKD who undergo hemodialysis commonly possess double lumen access, which makes them prone to bacteremia due to the nature of the procedure. Under pathogenic conditions, *Staphylococcus spp.* emerges as a leading cause of skin and soft tissue infections, including abscesses, furuncles, and cellulitis. *Staphylococcus aureus* is a prominent cause of

bacteremia, sepsis, and infective endocarditis. Additionally, it is responsible for osteoarticular infections, pneumonia, pleurisy, and device-associated infections, including those associated with double-lumen catheters and central venous catheters (Namvar et al. 2013, Tong et al. 2015).

Biofilm was formed in 83.3% of the samples in this study. Biofilm formation was observed in 66% of *Staphylococcus aureus* isolates, which was relevant to previous research. The majority of *Staphylococcus spp.* isolated from blood, urine, pus, and sputum samples were biofilm-forming isolates. In most studies, more than 50% of *Staphylococcus aureus* samples produced biofilm (Nourbakhsh & Namvar 2016, Neopane et al. 2018, Omidi et al. 2020). In a study conducted at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, a positive biofilm assay was seen in all (100%) MRSA carrier isolates. The study also revealed that 57.9% of clinical isolates produced biofilms according to the positive assay (Suryanditha et al. 2018). A study conducted at an Iranian hospital reported different findings. In the study, 46% of MRSA isolates produced strong biofilms on microtiter plates (Mirzaee et al. 2014).

In this study, it was found that 100% of coagulase-negative *Staphylococcus* isolates formed biofilms in vitro. This was in line with previous studies, which found that more than 90% of coagulase-negative *Staphylococcus* formed biofilms (Seng et al. 2017). Its ability to form biofilms was significantly stronger and more dominant than that of *Staphylococcus aureus* ($p<0.05$). Additionally, a previous study found that *Staphylococcus epidermidis* isolates showed a higher biofilm-forming capacity than *Staphylococcus aureus* isolates (Águila-Arcos et al. 2017).

The presence of methicillin-resistant coagulase-negative *Staphylococcus* (MR-ConS), which accounted for 83% of all coagulase-negative *Staphylococcus* samples, raised serious concern. This may escalate into a bigger problem if the person is immunocompromised, has a chronic illness such as diabetes, or uses medical devices. As the samples were obtained from patients, there was a high likelihood that the patients were infected with these bacteria. Moreover, the presence of biofilm would complicate treatment, exacerbating the bacterial resistance factor (Seng et al. 2017, Águila-Arcos et al. 2017, Piechota et al. 2018). Patients who use medical devices are more likely to develop biofilms, which lead to increased morbidity and mortality. Coagulase-negative *Staphylococcus* is also known as a contributing factor to sepsis in newborns and the elderly in developing countries (Kwiecinski et al. 2019, Ielapi et al. 2020, Singh et al. 2023). The formation of biofilms is unquestionably a contributing factor that complicates treatment for patients, often without proper notice. Through this simple study, it is hoped that clinicians can be aware of this virulence factor when treating patients.

Strength and limitations

This study used clinical isolates obtained from a hospital, where samples were taken from patients admitted with various diagnoses. Therefore, the findings were quite applicable in a clinical setting. However, a limitation of this study was the formation of in vitro biofilms, which may differ from in vivo biofilms due to differences in environmental conditions and host factors. Another limitation of this study was its limited scale.

CONCLUSION

The majority of *Staphylococcus aureus* and all coagulase-negative *Staphylococcus* isolates can produce biofilms in vitro. When compared, coagulase-negative *Staphylococcus* forms significantly more biofilm than *Staphylococcus aureus*. Clinicians are advised to incorporate practices such as using proper wound dressings and regularly replacing medical devices for patients. This approach aims to prevent the formation of biofilms and the unnecessary prolongation of antibiotic use. Furthermore, if there is a suspicion of infection, it is recommended to replace the medical devices as soon as possible, even before the scheduled treatment. Furthermore, commensal bacteria with low virulence factors should not be underestimated, especially when working with people who have compromised immune systems.

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Conflict of interest

None.

Ethical consideration

This study has received ethical approval from the Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Warmadewa, Denpasar, Indonesia, with registration No.

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Author contribution

MS contributed to the conception and design of this study, the analysis and interpretation of the data, and the drafting of the article. DAPSM contributed to the critical revision of the article for important intellectual content and final approval of the article. AAGI contributed to the analysis and interpretation of the data and provided statistical expertise. KS contributed to the provision of study materials as well as the collection and assembly of data. IKAIA contributed administrative, technical, and logistical support. MAbAR contributed technical advice.

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Original Research Report

FACTORS THAT CAUSE WORK FATIGUE AMONG SHIPYARD WORKERS

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ABSTRACT

Work fatigue remains a prevalent issue among workers in shipyards, leading to detrimental effects on physical well-being, including a decline in body resistance and productivity. The objective of this study was to analyze the relationship between age, nutritional status, working period, physical workload, noise, and lighting with fatigue levels among hull construction workers. This observational study used a cross-sectional approach with a total population of 112 individuals working in the production line of the Warship Division of a shipbuilding company. The sample size in this study was determined using stratified random sampling, resulting in 53 samples. Proportionate stratified random sampling was then employed to determine the sample size for each subgroup of the population. The research data were analyzed using the Spearman correlation test ($p<0.005$). This study found a significant relationship between work fatigue and nutritional status ($p=0.000$), with a moderately positive relationship ($r=0.511$). Additionally, there was a significant relationship between work fatigue and physical workload ($p=0.000$), with a moderately positive relationship ($r=0.531$). Noise and work fatigue showed a significant relationship ($p=0.010$), with a weak positive relationship ($r=0.353$). Lighting and work fatigue showed a significant relationship as well ($p=0.000$), but with a moderately negative relationship ($r=-0.524$). On the other hand, work fatigue did not exhibit any significant relationship with age ($p=0.129$) or working period ($p=0.651$). This study found a relationship between work fatigue and numerous factors, including nutritional status, physical workload, noise, and lighting. However, age and working period were not related to work fatigue. Thus, it is recommended that companies align job requirements with employees' work capacity and conduct regular assessments of noise and lighting conditions to mitigate work-related fatigue.

Keywords: Nutritional status; physical workload; noise; work fatigue; public health

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Highlights:
1. The focus of this study was on the underlying factors of fatigue among shipyard workers, an area that necessitates further research to attain ideal standards in occupational health and safety.
2. This study can provide companies with material to use when counseling workers on the importance of a healthy nutritional intake in preventing fatigue.

INTRODUCTION

All workers have the right to assurances regarding their health and safety at work, according to the Law of the Republic of Indonesia Number 13 of 2003 Concerning Manpower. Therefore, it is the responsibility of the company to establish and implement an occupational health and safety program to protect workers from potential hazards during the course of their duties. The employment report of the Social Security Implementation Agency documented workplace accidents in Indonesia that have been increasing in recent years. A total of 123,041 work-related accidents were reported in 2017, while an increase to 173,105 was recorded in 2018. The agency handles an annual average of 130,000 work-related accidents, ranging from minor mishaps to fatalities (Monalisa et al. 2022).

One of the most unsafe conditions for workers is fatigue. Work fatigue is a physical and psychological condition that reduces an individual's workability and resistance to activities. It can harm workers by causing accidents, injuries, and decreased work productivity (Suma'mur 2014). According to the report of the Intenational Labour Organization (2018), work fatigue was responsible for the deaths of nearly two million workers. The results from their survey of 58,118 samples revealed that approximately 32.8% of workers, or 18,828 individuals, had experienced work fatigue. The findings of a previous study provided additional evidence for the prevalence of work fatigue. It was found that a total of 23–40% of workers experienced high levels of work fatigue (Tung & Hsiung 2021).

Work fatigue can affect workers in any sector, including those working in shipyards. There are approximately 250 shipyards in Indonesia, each employing a diverse range of workers. According to a previous study, 56.5% of workers suffer from work fatigue. Workers who endure such fatigue are more at risk of experiencing work-related accidents. In addition, a separate study revealed comparable findings, showing that 56.2% of shipyard workers suffered from work fatigue (Pratiwi 2019).

Work fatigue may arise due to internal factors including but not limited to age, nutritional status, years of service, and gender. However, in addition to internal factors, work fatigue may also result from external factors, such as the physical work environment, workload, length of working time, and monotony (Suma'mur 2014). A study conducted by Larasati & Puspitasari (2019) showed that a significant proportion of workers (82.8%) who had a relatively heavy physical workload were found to experience high levels of work fatigue. The physical work environment, including factors such as lighting and noise, can also contribute to work fatigue.

The workday of shipyard workers in Indonesia starts early at 7.30 a.m. and concludes at 4.30 p.m. Most of the workers' activities aboard the ship take place while the ship is still in the construction process. Consequently, the workers rely solely on substandard streetlamps for lighting. In addition, the workers are frequently exposed to noise generated from the tools they use in their work, such as hammers, grinders, rotary impact drills, welding machines, diesel generators, and blowers. They also use more muscular energy to complete their work (Kaunang et al. 2019). The aforementioned working conditions sparked the authors' interest regarding work fatigue among shipyard workers. Therefore, this study aimed to analyze the factors that contribute to work fatigue among shipyard workers, particularly in the warship division of a shipbuilding company in Surabaya, Indonesia, where there were four ship projects in progress at the time of this research.

MATERIALS AND METHODS

This was an analytical observational study in which data were collected solely from research subjects without providing any intervention. This study used a cross-sectional design because the research was conducted at a certain time (Spector 2019). The ethical approval for this study was issued by the Health Research Ethics Committee of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia (No. 032/HRECC.FODM/2022 on 26/1 /2022). The research was conducted in March 2022 at the warship division of a shipbuilding company in Surabaya, Indonesia.

This study used stratified random sampling to determine the required research sample size. A total of 53 samples were acquired according to the sampling technique. Afterwards, this study used proportionate stratified random sampling to determine the sample size for each of the three workshops, i.e., the Hull Construction Workshop, the Machinery Outfitting Workshop, and the Hull Outfitting Workshop (Etikan 2017). The obtained samples were 21 workers from the Hull Construction Workshop, 20 workers from the Machinery Outfitting Workshop, and 12 workers from the Hull Outfitting Workshop. The variables analyzed in this study were independent and dependent variables. The independent variables consisted of age, nutritional status, years of service, physical workload, noise exposure, and lighting. Meanwhile, work fatigue served as the dependent variable.

The nutritional status variable was determined by initially measuring the workers' weight and height using a digital scale and a microtome, respectively. Afterwards, the body mass index (BMI) formula was calculated to determine the nutritional status. The measurement of physical workload was carried out using the percentage of cardiovascular load (%CVL) method by recording the workers' pulse. The measurement of work fatigue was conducted by using a reaction timer on a sample of 53 shipyard workers (Halson 2014). All data were statistically analyzed using IBM SPSS Statistics for Windows version 25.0 (IBM Corp., Armonk, N.Y., USA). The technique used for data analysis was Spearman's correlation coefficient ($p<0.05$). The analysis assessed the monotonic function, a function that is in accordance with the arbitrary, to describe the relationship between two variables without making any assumptions regarding the frequency distribution of the variables being examined (Yasril & Fatma 2021).

RESULTS

The present study included a sample of 53 individuals who were employed as shipyard workers. According to the data presented in Table 1, it is evident that the majority of workers are within the age group of 35 years, which includes a total of 27 individuals (51%). Most of the workers had a moderate degree of underweight in terms of their nutritional status, as shown by a total of 15 workers (28.3%). In addition, a substantial proportion of the workers, comprising 33 individuals (62.3%), had been working for a period of 6–10 years.

Table 1. Distribution of characteristics of the shipyard workers (n=53).

Variables	Categories	n	%
Age	35 years old	27	51.0
	36–50 years old	21	39.6
	>50 years old	5	9.4
Nutritional status	Severely underweight	9	17.0
	Moderately underweight	10	18.9
	Normal	12	22.6
	Overweight	15	28.3
	Obese	7	13.2
Working period	5 years	8	15.1
	6–10 years	33	62.3
	>10 years	12	22.6
Physical workload	Light	5	9.4
	Medium	15	28.3
	Slightly heavy	23	43.4
	Heavy	7	13.2
	Very heavy	3	5.7
Noise	<PEL	14	26.4
	>PEL	39	73.6
Lighting	Standard	33	62.3
	Substandard	20	37.7
Work fatigue	Normal	30	56.6
	Mild	22	41.5
	Moderate	1	1.9

PEL: Permissible exposure limit.

This study revealed that nearly half of the workers, specifically 23 individuals (43.4%), exerted themselves in physical tasks that fell within the slightly heavy workload category. Regarding the working environment in the shipyard, the analysis focused on two factors contributing to work fatigue, i.e., noise and lighting conditions. It was found that a majority of workers performed their tasks in areas where noise levels exceeded the permissible exposure limit, encompassing a total of 39 individuals (73.6%). Moreover, a large portion of workers carried out their tasks with substandard lighting, including 33 individuals (62.3%). With regard to the aforementioned factors pertaining to the workers and their working environment, it was observed that they exhibited varying degrees of work fatigue. Nevertheless, a significant proportion of workers experienced work fatigue that was still within the normal category, with as many as 30 workers (56.6%).

The data on the workers' characteristics were then analyzed by cross-tabulation to examine the relationship between work fatigue and several factors, including age, nutritional status, working period, physical workload, noise, and lighting (Table 2). Although work fatigue in the normal category was the most prevalent, more than half of workers aged 35 exhibited mild work fatigue. A total of 14 workers in the age group of 35 years old experienced mild work fatigue, which accounted for 51.9% of the total sample in this group. However, the Spearman correlation test yielded a p-value of 0.129, suggesting a lack of a significant relationship between age and work fatigue among shipyard workers.

A substantial majority of workers with an overweight status had complaints of mild work fatigue, with as many as 10 individuals (66.7%). The Spearman correlation test analysis yielded a p-value of 0.000, showing a statistically significant relationship between nutritional status and work fatigue among shipyard workers. Additionally, the Spearman correlation analysis produced a correlation coefficient of 0.511, indicating a moderately positive or unidirectional relationship between these two variables.

The majority of workers with a working period ranging from 6 to 10 years stated having mild work fatigue. This was observed in 17 individuals, accounting for 51.5% of the subgroup size. The results of the Spearman correlation test revealed a p-value of 0.651, indicating the absence of a significant relationship between the working period and work fatigue experienced by the shipyard workers.

There was a concern that work fatigue was caused by an intense physical workload. The results of this study showed that the majority of workers had a slightly heavy physical workload, accompanied by a relatively mild degree of work fatigue. This was demonstrated by a total of 16 individuals, accounting for 69.6% of the subgroup size. The Spearman correlation test found a p-value of 0.000, indicating a statistically significant relationship between physical workload and work fatigue among shipyard workers. In addition, the obtained correlation coefficient of 0.531 indicated a moderate level of relationship between the two variables.

Table 2. Relationship between work fatigue and its underlying factors among the shipyard workers (n=53).

Variables	Categories	Work fatigue						p
		Normal		Mild		Moderate		
		n	%	n	%	n	%	
Age	35 years old	13	48.1	14	51.9	0	0	
	36–50 years old	12	57.1	8	38.1	1	4.8	
	>50 years old	5	100	0	0	0	0	
Total		30	56.6	22	41.5	1	1.9	0.129
Nutritional status	Severely underweight	7	77.8	2	22.2	0	0	
	Moderately underweight	9	90.0	1	10.0	0	0	
	Normal	8	66.7	4	33.3	0	0	
	Overweight	5	33.3	10	66.7	0	0	
	Obese	1	14.3	5	71.4	1	14.3	
Total		30	56.6	22	41.5	1	1.9	0.000
Working period	5 years	7	87.5	1	12.5	0	0	
	6–10 years	15	45.5	17	51.5	1	3	
	>10 years	8	66.7	4	33.3	0	0	
Total		30	56.6	22	41.5	1	1.9	0.651
Physical workload	Light	5	100	0	0	0	0	
	Medium	14	93.3	1	6.7	0	0	
	Slightly heavy	7	30.4	16	69.6	0	0	
	Heavy	3	42.9	4	57.1	0	0	
	Very heavy	1	33.3	1	33.3	1	33.3	
Total		30	56.6	22	41.5	1	1.9	0.000
Noise	<PEL	12	85.7	2	14.3	0	0	
	>PEL	18	46.1	20	51.3	1	2.6	
Total		30	56.6	22	41.5	1	1.9	0.010
Lighting	Standard	12	36.4	20	60.6	1	3	
	Substandard	18	90.0	2	10.0	0	0	
Total		30	56.6	22	41.5	1	1.9	0.010

A substantial number of workers employed in areas that had excessive noise levels beyond the permissible exposure limit experienced mild work fatigue, with as many as 20 individuals (51.3%). The Spearman correlation test resulted in a p-value of 0.010. Hence, there was a significant relationship between noise and work fatigue among shipyard workers. Furthermore, the obtained correlation coefficient of 0.353 indicated that the two variables had a weak positive relationship.

Most of the workers (60.6%) who carried out their activities in areas with inadequate lighting experienced mild work fatigue. The results of the Spearman correlation test showed a p-value of 0.000. Therefore, a significant relationship was observed between lighting and work fatigue experienced by shipyard workers. The obtained correlation coefficient of -0.524 showed a moderate degree of relationship between lighting and work fatigue. It was implied that higher lighting intensity was necessary to lower the level of work fatigue.

DISCUSSION

Work fatigue is a condition characterized by a decline in work capacity and physical strength, resulting in a diminished ability to sustain work performance. Work fatigue also includes declined physical exertion, feelings of exhaustion, decreased motivation, and poor work productivity (Suma'mur 2014). According to the findings of this study, it was evident that the majority of the shipyard workers did not have work fatigue. However, this might be due to the relatively low work intensity observed throughout the period of the research, particularly in the Hull Construction Workshop and the Electric Outfitting and Interior Workshop.

The prevalence of mild work fatigue was mostly observed among workers in the Machinery Outfitting Workshop and the Hull Outfitting Workshop. This was attributed to the time constraints imposed on the workshops to finish the targets within a specified timeframe during the period of this study, resulting in an intense workload for the workers. The workshops frequently failed to achieve their targets, requiring the workers to subsequently revise their work. In addition, workers in the Machinery Outfitting Workshop and the Hull Outfitting Workshop often experience occupational accidents, including minor injuries such as scratches and bumps. Failure to immediately manage work fatigue can lead to a decline in work motivation and performance, as well as an increased likelihood of making mistakes, decreased productivity, job-related injuries, and work accidents (Tarwaka 2019).

Relationship between age and work fatigue among shipyard workers

Among the 53 workers examined in this study, 27 (51.0%) were 35 years old, placing them within the

age range that is considered productive. These workers have higher productivity compared to older workers. This is due to the fact that as workers age, their physical condition deteriorates and their mobility becomes limited (Aprilyanti 2017). Age is an individual factor that may influence the level of work fatigue. With increasing age, muscle strength and functional capacity will diminish, making workers more susceptible to fatigue (Suma'mur 2014). However, the findings of this study revealed that there was no relationship between age and work fatigue among shipyard workers. This was in line with previous research that concluded there was no relationship between the two variables in the context of gold mining production workers (Wahyuni & Indriyani 2019). In addition, no statistically significant relationship was found between age and work fatigue in a separate study. It was determined that there was no relationship between age and fatigue among ceramic production workers (Juliana et al. 2018).

The majority of subjects in this study who reported mild fatigue were 35-year-old workers. The young workers were more likely to experience mild work fatigue because a lot of workers over the age of 35 acted as group leaders and therefore monitored work more. Conversely, the 35-year-old workers were assigned a greater number of duties, while the group leaders verified the outcomes. The research findings of Dewi et al. (2019) showed that younger workers are more prone to experiencing work fatigue. Younger workers are generally perceived to have superior physical conditions than their older colleagues, leading to increased workloads and extended work hours.

Relationship between nutritional status and work fatigue among shipyard workers

As many as 15 individuals (28.3%) among 53 workers were overweight in terms of their nutritional status. The measurement of the workers' nutritional status in this study was conducted using BMI, which was determined by their height and weight (Suma'mur 2014). In the meantime, the body requires balanced nutrition to support physical growth, brain development, and workability. The role of nutrition is to support workers in their activities by providing the necessary energy to mitigate the risk of work fatigue. Therefore, healthy nutritional status can prevent fatigue and improve the overall health of workers (Garedja et al. 2017, Natizatun et al. 2018).

The findings of this study revealed a statistically significant relationship between nutritional status and work fatigue. This observation aligns with other studies that have demonstrated a relationship between nutritional status and work fatigue (Safira et al. 2020). The studies similarly identified a relationship between the two variables. Nevertheless, the analysis revealed that the relationship between the variables was of moderate significance (Amin et al. 2019).

In this study, most of the workers who were overweight experienced mild fatigue. This was due to their preference for buying meals from outside merchants rather than consuming the food provided by the company. In addition, it is worth noting that there was a lack of guidance from the company on the importance of maintaining a balanced diet as a means to prevent work fatigue. Excess fat may limit the ability of the workers' muscles and bones to perform physical tasks. The accumulation of excess fat within the vital organs of the body can lead to a decline in organ functionality and an inability to effectively meet occupational needs, hence causing workers to suffer from work fatigue (Tarwaka 2019).

Relationship between working period and work fatigue among shipyard workers

The working period refers to the duration during which a worker has been engaged in carrying out their duties within an annual period. The factor of working period in this study was the length of time the workers had been performing their duties, specifically up until the research was conducted. The majority of the workers had a working period ranging from 6 to 10 years, accounting for 33 individuals, or 62.3% of the total sample. Workers with a long working period are less likely to experience fatigue due to their ability to adapt to their jobs and work environment, as well as their level of experience in doing their tasks (Suma'mur 2014).

In this study, there was no significant relationship between working period and work fatigue among shipyard workers. This finding is consistent with previous research, which indicated a lack of relationship between working period and fatigue (Yamaula et al. 2021). In a separate study, a comparable result was discovered. The research showed no significant relationship between working period and work fatigue (Malik et al. 2021).

A large number of young workers have work experience over five years, primarily due to their participation in employment after graduating from high school. Therefore, even though they are relatively young, they are included in the category of individuals with a long working period. While previous research suggested that individuals with a long working period are less likely to experience fatigue, a separate study presented contrasting findings. Workers with long working periods may

potentially experience higher levels of fatigue as a result of the monotonous nature of their jobs (Asriyani & Karimuna 2017).

Relationship between physical workload and work fatigue among shipyard workers

The physical workload serves as a representation of the requirement that the company imposes, requiring the use of physical energy to complete the assigned tasks. All physical activities carried out by workers are considered workload. Physical workload can affect bodily organs, including changes in heart rate (Tarwaka 2019). Workers may have a heavy physical workload due to the daily requirement of achieving targets on the same day. Shipyard workers in this study mostly had a slightly heavy physical workload, as shown by 23 individuals (43.4%). The findings of this study indicated a statistically significant relationship between physical workload and work fatigue. The workers who had a slightly heavy physical workload were primarily employed in the Machinery Outfitting Workshop and Hull Outfitting Workshop. This might be attributed to the inherent demands of the assigned tasks, which must be completed as part of specified targets. Additionally, the need for repeated revisions due to suboptimal work quality further contributed to the physical workload. The operation of equipment and the handling of materials required the workers' extra energy. On a daily basis, workers were assigned a certain target that needed to be accomplished within the same day. In instances where the assigned tasks were uncompleted, workers were obligated to work overtime, resulting in an increase in the physical workload.

The research conducted by Kaunang et al. (2019) yielded comparable results to the present study. A relationship was discovered between physical workload and work fatigue. This study is consistent with the findings of Pua et al. (2020), who found a moderately positive correlation between physical workload and work fatigue. The physical workload may increase when tasks are carried out manually. Shipyard workers are classified as manual laborers due to their role as operators of various equipment, including grinding, hammering, welding, and cutting. The physical workload placed on workers can increase when they lack optimal nutrition and are exposed to high noise levels and inadequate lighting conditions (Sakti 2021). When workers' workload surpasses their capacity, it leads to a decline in muscle strength for contraction. This results in muscle weakness and subsequently causes work fatigue (Pujiastuti et al. 2021).

Relationship between noise and work fatigue among shipyard workers

Excessive noise in the workplace can have detrimental effects on workers. It may lead to many issues, such as communication problems, compromised health, decreased concentration, and increased fatigue (Suma'mur, 2014). The current study conducted measurements of noise levels at ten specific locations throughout four different ships. The results revealed that eight of these measurement points exceeded the permissible exposure limit, while the other two locations did not surpass the limit. The permissible exposure limit for noise was set at 85 dBA, with a daily exposure duration of eight hours (Ministry of Manpower of Republic Indonesia 2018). In these ten locations, equipment was the main source of noise. These included hammers, rotary impact drills, welding machines, diesel generators, cable cutters, air conditioners, and blowers (Sudirman Central Business District 2017).

The company in which this study was conducted had never measured noise levels on the ship, despite the majority of workers undertaking their duties aboard. Some workers were exposed to noise exceeding the permissible exposure limit without using personal protective equipment, such as ear plugs or ear muffs, indicating a lack of initiative in reducing noise exposure. Workers who are exposed to noise levels over 85 dBA are required to wear personal protective equipment to prevent their exposure from exceeding the permissible exposure limit. In addition to the use of personal protective equipment, work rotation may help minimize high noise exposure that causes health problems (Suma'mur 2014). In this study, it was found that there was a statistically significant relationship between noise and work fatigue among shipyard workers. This finding is in line with prior research that has reported a weak relationship between noise and work fatigue (Rahmawati & Tualeka, 2019). The impact of noise on workers' performance and well-being has been investigated as an essential work environment factor. A separate study resulted in the finding of a positive relationship between noise and work fatigue (Pratiwi 2019, Kurniawan et al. 2020).

Silencers were not provided for the machinery used by the workers in this study. Due to the narrow space of the ships, the intensity of the noise escalated. Excessive noise in work environments has been observed to have detrimental effects on workers' concentration and performance. It may lead the workers to become careless and make mistakes, indicating a symptom of work fatigue. The central nervous system is made up of an activation system, also known as the drive system, and an inhibition system. These two systems function in an alternating manner to regulate various processes within the

brain. In environments with a high level of noise, the thalamus exerts a stimulating effect on the inhibition system, hence strengthening its activity (Suma'mur 2014). Consequently, workers exposed to such conditions may exhibit symptoms of body weakness and work fatigue.

Relationship between lighting and work fatigue among shipyard workers

The provision of adequate lighting is an important factor for workers since it allows them to clearly see objects with less eyestrain. The lighting in the work area should be adjusted to adequately meet the requirements—neither too little nor too much (Soeprapto et al. 2021). Work fatigue and decreased concentration may occur as a result of inadequate lighting required for seeing objects, while excessive lighting can result in glare. The recommended lighting intensity for tasks involving the visual identification of small objects, such as iron and steel, is set at 100 lux (Syekura & Febriyanto 2021).

This study conducted lighting measurements at ten specific locations on four fast missile boats. The lighting within the ships failed to adhere to the established standards because the lamps were used alternately, resulting in certain areas not having adequate lighting. Additionally, the vessel lacked ventilation, thus limiting natural lighting in some work areas (Ministry of Manpower of Republic Indonesia 2018). There was only a single streetlamp on the ship, and it moved around. The lamp's brightness was insufficient to adequately illuminate the entire space. Within the ships, there were three distinct tasks undergoing at different work areas. As a result, the workers who were positioned with their backs toward the light source experienced inadequate lighting. Due to the inadequate lighting conditions in the work areas, the workers' visual perception was compromised, necessitating increased eyestrain to clearly see objects. Symptoms that arise due to excessive eyestrain include headaches, decreased cognitive skills, and decreased concentration. These symptoms are indications of work-related fatigue (Suma'mur 2014).

The findings of this study revealed a statistically significant relationship between lighting and work fatigue among shipyard workers. As a result, the findings emphasize the importance of ensuring adequate illumination to mitigate the workers' fatigue. Previous studies showed similar results regarding the two variables (Yogisutanti et al. 2020, Adventina & Widanarko 2021). A negative correlation was observed between lighting and work fatigue. This indicates that a lesser intensity of lighting leads to a higher level of work fatigue.

Strength and limitations

One notable aspect of this study is its novel approach to the measurements of noise and lighting that had not been previously documented at the research site. Moreover, this study may also motivate companies to conduct routine measurements to prevent the development of occupational diseases. However, the limitation of this study was that the data were collected during a period of low work intensity, thereby limiting the measurement of work fatigue.

CONCLUSION

A relationship was found between shipyard workers' work fatigue and other factors, including nutritional status, physical workload, noise, and lighting. In contrast, shipyard workers' work fatigue did not exhibit a relationship with either age or working period. Companies should provide counseling services concerning the importance of healthy nutritional intake. Additionally, it is recommended that companies adjust workloads in accordance with their workers' capacity, conduct regular measurements of noise and lighting levels on board at least once annually, and monitor the equipment required for work on board, such as streetlamps and engine silencers. Lastly, companies need to enforce strict adherence to the use of personal protective equipment as a means of mitigating the risk of work fatigue.

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Conflict of interest

None.

Ethical consideration

This study was approved by the Health Research Ethics Committee of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia (No. 032/HRECC.FODM/I/2022 on 26/1/2022).

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Author contribution

SR contributed to the drafting and critical revision

of the article for important intellectual content. NW contributed to the final approval of the article and the provision of study materials. ED contributed to the analysis and interpretation of the data and the final approval of the article. SME contributed to the critical revision of the article for important intellectual content and statistical expertise. AAE contributed to the drafting of the article and the provision of administrative, technical, and logistic support. ANA contributed to the conception and design, as well as the collection and assembly of data.

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Original Research Report

ANTIBIOTIC-PRODUCING *Streptomyces sp.* ISOLATED FROM THE SOIL OF A MANGROVE ECOSYSTEM

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ABSTRACT

A mangrove ecosystem in Surabaya, Indonesia, has a high salinity, pH, potassium, phosphorus, and nitrate contents. This ecosystem comprises a mixture of sand, dust, mud, and clay, which has the potential to be a conducive environment for the isolation of *Streptomyces*. The importance of *Streptomyces* in biotechnology lies in its ability to produce bioactive secondary metabolites, which represent a valuable reservoir of antibiotics. This study aimed to assess the antibiotic activity exhibited by *Streptomyces* sp. isolated from the soil of a mangrove ecosystem in Wonorejo, Surabaya, Indonesia. The analysis focused on the potential of *Streptomyces* sp. to produce antibiotics that work against Gram-positive bacteria (i.e., *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis*) as well as Gram-negative bacteria (i.e., *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella Typhimurium*). The antibacterial activity test was conducted using the modified agar diffusion method. Observations were performed to identify any clear zone formation around the *Streptomyces* sp. agar colonies with a diameter of 0.8 cm and a height of 3 mm. The clear zone diameter was measured every 24 hours during the 10-day incubation period to assess the diversity of antibacterial activity. The antibacterial profile of *Streptomyces* sp. exhibited varying levels of activity against different bacterial strains in the tests conducted. The inhibition zone diameters demonstrated the highest levels of activity in *Bacillus subtilis* (15.9 mm) on day 7, *Staphylococcus aureus* (27.6 mm) on day 2, *Pseudomonas aeruginosa* (24.3 mm) on day 7, *Escherichia coli* (29.2 mm) on day 5, and *Salmonella Typhimurium* (27.5 mm) on day 7. The results indicated that *Streptomyces* sp. had inhibitory effects against Gram-positive bacteria as well as Gram-negative bacteria. In conclusion, *Streptomyces* sp. found in the soil of mangrove ecosystems has the ability to produce antibiotics.

Keywords: *Streptomyces* sp.; mangrove; antibiotics; biodiversity; good health and well-being

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Highlights:
1. The unexplored soil of mangrove ecosystems in Surabaya, Indonesia, has the potential to be home to biodiversity, including *Streptomyces* sp. that can produce antibiotics.
2. *Streptomyces* sp. has antibacterial properties against Gram-positive and Gram-negative bacteria, and the duration of incubation plays a critical role in regulating the antibacterial activity.

INTRODUCTION

Mangrove forests can absorb contaminants originating from upstream areas through rivers and surrounding dry lands through rainwater. These contaminants contain various compounds, such as ammonia, nitrate, and nitrite, which serve as nutrients for decomposing bacteria (Chrisyariati et al. 2014, Alongi 2020). The structure and composition of a mangrove ecosystem have a direct correlation with its nutrient profile. The decrease in nutrient content of the ecosystem is expected to have a negative impact on mangrove growth. Nitrogen and phosphorus are the basic inorganic nutrients that are essential for the process of primary production. The ideal nutrient profiles of mangrove vegetation

are 0.211–0.252 mg/L for nitrate, 0.135–0.277 mg/L for nitrite, 0.51–0.74 mg/L for ammonia, and 0.109–0.140 mg/L for phosphate (Frederika et al. 2021).

Mangrove ecosystems are categorized based on their biophysical types. The categorization includes numerous types of mangroves, i.e., deltaic, estuarine, lagoonal, and open-coast mangroves (Worthington et al. 2020). A study conducted by Constance et al. (2022) examined the lagoonal mangrove ecosystem located in Aldabra Atoll, Seychelles. The findings of the study estimated an average aboveground biomass (AGB) of 82±13 Mg ha^{-1}. In another study, Palla et al. (2018) conducted research that examined tropical mangrove forests. The researchers found a microbial composition consisting of 91% bacteria and fungi, 7% algae, and 2% protozoa.

Previous research has documented the presence of several bacterial strains, such as *Streptomyces*, within mangrove ecosystems. *Streptomyces* is a Gram-positive, rod-shaped, filamentous bacterium characterized by its hyphae diameter of 0.5–1.0 mm. It has an aerobic microbial life and a diaminopimelic cell wall. In contrast to the rapid growth observed in other bacterial colonies, *Streptomyces* colonies present with a slow emergence and strong adhesion to agar media. These colonies have distinct clusters or granules in liquid culture media, with their characteristics highlighted by their opaque appearance (Chater 2016, Al-Dhabi et al. 2018, 2019). The growth of *Streptomyces* begins with the germination of the spores and the development of hyphae. Afterwards, the primary and secondary mycelia start to form, leading to the development of unigenomic spores at the end of the cycle. The secondary mycelium is responsible for the expression of genes or proteins that are involved in secondary metabolism, both in solid and liquid cultures (Manteca & Yagüe 2018).

Streptomyces, a microorganism that was formerly misidentified as a fungus, infrequently induces pathogenic conditions. The microorganism is characterized by its filamentous structure and sporulation capability. It has a notable guanine + cytosine (G+C) content of 73%, which is higher than many other organisms. *Streptomyces* can thrive within a pH range of 3–9 and a temperature of 15–45°C. The optimal growth process for *Streptomyces* is feasible at normal room temperature. However, the growth of *Streptomyces* is strongly influenced by pH levels. The growth rate decreased significantly at pH levels of 3 and 9. The ideal pH level for achieving maximum growth of *Streptomyces* sp. is 7 (Baskaran & Muthukumarasamy 2017).

Streptomyces remains one of the main natural sources for antibiotics and several bioactive compounds. It plays an important role as the primary producer of approximately two-thirds of antibiotics in the fields of medicine and agriculture. However, the secondary metabolic pathways provided by *Streptomyces* have not been explored enough in recent laboratory culture research (Manteca & Yagüe 2018, Karthik & Kalyani 2023). When conducting research on *Streptomyces*, nitrogen concentration serves as an indicator of its presence within mangrove ecosystems. One of the characteristics of *Streptomyces* is its ability to enzymatically convert nitrate compounds into nitrite. Therefore, differences in mangrove soil texture can potentially influence the nitrogen concentration, which in turn acts as a reliable marker for the existence of various kinds of *Streptomyces* bacteria (Madigan et al. 2019).

Water pollution poses harmful effects on human individuals and populations, as well as on mangrove ecosystems. However, some organisms can flourish even in the presence of polluted waste, thereby contributing to the overall biodiversity of these ecosystems. The *Streptopmyces* bacteria that survive in contaminated mangrove ecosystems are considered distinct isolates (Al-Ansari et al. 2019). Instances of water pollution frequently arise in Surabaya, Indonesia, particularly along the rivers and mangrove forests, with a notable concentration downstream of ponds in the estuaries and coastal areas. The area with the widest and most diverse mangrove ecosystem along the Surabaya shoreline is located on the east coast of the city (Syamsu et al. 2018, Sukojo & Arindi 2019). Hence, this study aimed to assess the antibiotic profile of *Streptomyces* sp. isolates obtained from the mangrove ecosystem soil in Wonorejo, Surabaya, Indonesia. The assessment focused on the activity of these isolates against both Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

The technique used was a modified agar diffusion method. Agar molds were made every 24 hours for ten days using *Streptomyces* sp. which was 0.8 cm in diameter and 3 mm high. To test potential, the test object was then inserted into the test medium. Agar mold containing *Streptomyces* sp. attached to culture media that has previously been inoculated with test bacteria to carry out antibacterial activity tests. The media used in this study was ISP-4 (International *Streptomyces* Project medium 4).

Microscopic identification of *Streptomyces* sp.

The *Streptomyces* sp. bacteria were grown on International *Streptomyces* Project 4 (ISP-4) medium, with catalog number 277210, produced by Difco Laboratories (Detroit, MI, USA). The

incubation period was carried out for four days at a temperature of 28°C. Microscopic observation was conducted by transferring a loopful of *Streptomyces* sp. using a sterile inoculation loop into an object glass filled with sterile water, then examining it under an Olympus U-5RE-2 microscope (Munday Scientific, Sanford, NC, USA) with 400X magnification by adding immersion oil (Girkin 2019).

Preparation of *Streptomyces* sp. cell suspension

The preparation of *Streptomyces* sp. cell suspension started by adding 10 mL of sterile phosphate buffer solution (product number P3228, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) with a pH of 7 to ISP-4 agar slants (product number BD 277210, Becton Drive, Franklin Lakes, NJ, USA) containing *Streptomyces* sp. obtained from the soil of a mangrove ecosystem in Wonorejo, Surabaya, Indonesia (-7.305096, 112.843754), which had been incubated at 30°C for 24 hours. Subsequently, the agar slants were shaken until all colonies on the agar surface were released and suspended in a phosphate buffer with a pH level of 7. The inoculum was then measured at a transmittance of 25% with a wavelength of 580 nm using a Spectronic 20 (Thermo Fisher Scientific, Waltham, MA, USA). Afterwards, 5 mL of cell suspension was mixed with 15 mL of ISP-4 agar media. The agar media had been thawed at a temperature of 45°C and subsequently incubated at 28°C. The agar print, consisting of *Streptomyces* sp. with a diameter of 0.8 cm and a height of 3 mm, was taken every 24 hours for 10 days. The specimens were then placed on the test medium for potency testing (Rütten et al. 2022).

Antibacterial bioassay of *Streptomyces* sp.

The *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella Typhimurium* strains used in this study were sourced from the Microbiology Laboratory of the Department of Medical Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. The bacteria were cultured in 10 mL of nutritional agar slant media and incubated at a temperature of 30°C for 24 hours. Petri dishes were prepared by adding 15 mL of nutrient agar medium (Oxoid CM0003B, Oxoid Ltd., Basingstoke, UK) as a foundational layer. A volume of 10 µL of *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella Typhimurium* was added to each petri dish as a seed coating. The bacteria had a transmittance (T) of 25% measured using a spectrophotometer (Spectronic 20, Thermo Fisher Scientific, Waltham, MA, USA). The antibacterial activity test was carried out by attaching an agar print containing *Streptomyces* sp. onto the culture medium that had been previously inoculated with the test bacteria. Afterwards, the specimens were incubated at a temperature of 28°C for 24 hours. The measurement of the inhibition zone around the cultured colonies was conducted using a caliper. A volume of 10 µL of streptomycin sulfate solution with a concentration of 250 ppm was added to a well with dimensions of 0.8 cm in diameter and 3 mm in height. This study used positive and negative control groups. The positive control group contained antibiotic streptomycin sulfate on the test bacteria, while the negative control group contained sterile phosphate buffer. The positive control showed the presence of an inhibition zone around the cultured petri dish, while the negative control showed no inhibition zone at all. The presence of an inhibition zone surrounding the culture colonies on the petri dish was indicative of a positive result, suggesting that the *Streptomyces* sp. isolates could produce antibiotics by inhibiting bacterial growth. The antibacterial activity test of *Streptomyces* sp. was conducted in three replications (Al-Ansari et al. 2019).

RESULTS

After being incubated at a temperature of 28°C for two days, *Streptomyces* sp. isolates obtained from the soil of the mangrove ecosystem in Surabaya, Indonesia, exhibited distinct olfactory characteristics reminiscent of soil, along with the presence of small colonies. The appearance of the colonies' surface was initially observed on day 2, with a relatively smooth texture. The surface of the colonies had formed completely by day 4. The granularity of mycelium was also revealed during the observation. The colonies of *Streptomyces* sp. that were 14 days old showed several distinct characteristics. They were white, dry, and turbid, with no exudate present. The colonies had a circular shape and a convex surface that was not translucent. The growth of the colonies was slow, and they had rather thick spores, as seen in Figure 1.

The results of the antimicrobial activity test indicated that the *Streptomyces* sp. isolates exhibited effects against both Gram-positive bacteria (i.e., *Bacillus subtilis* and *Staphylococcus aureus* ATCC 25923) and Gram-negative bacteria (i.e., *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella Typhimurium*). This activity was evidenced by the formation of inhibition zones. The observed activity against the tested bacteria varied in terms of the initial detection time, the diameter of the inhibition zones, the duration of inhibitory activity, and the time required to reach the maximum production of antibiotics.

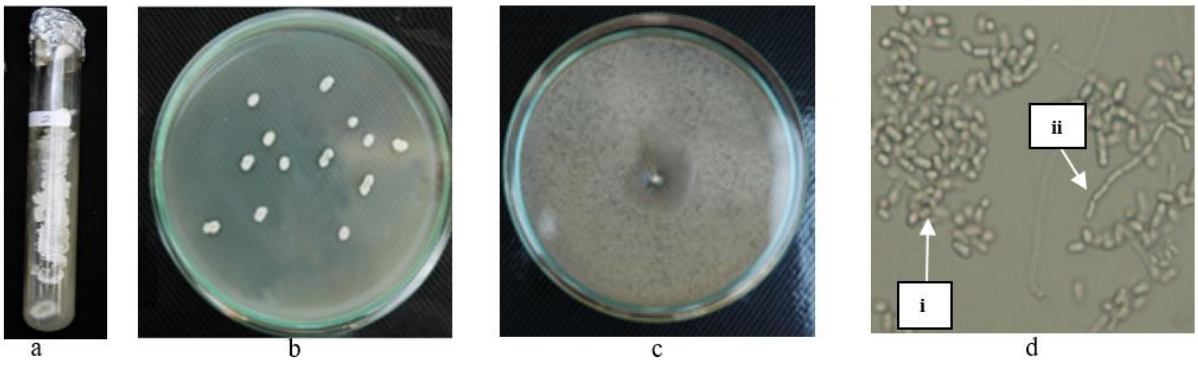

Figure 1. The characteristics of *Streptomyces* sp. through macroscopic and microscopic observations.
Notes: (a, b) Characteristics of *Streptomyces* sp. shown in the macroscopic observation. (c) Screening of the antibacterial activity of *Streptomyces* sp. against *Bacillus subtilis* using diffusion method. (d) Microscopic observation showing the characteristics of *Streptomyces* sp., specifically spore (i) and hyphae (ii).

The *Streptomyces* sp. isolates showed antibacterial activity against *Bacillus subtilis* starting from day 2 until day 7. The peak of the activity was seen on day 7, as evidenced by the largest inhibition zone diameter of 15.9 mm. Between days 8 and 10, a decline in activity was observed, as indicated by the reduced diameter of the inhibition zone. On day 10, the inhibition zone reached its smallest diameter of 11 mm.

In the test against *Staphylococcus aureus* ATCC 25923, the *Streptomyces* sp. isolates showed antibacterial activity, which started on day 2 and reached its peak on the same day. This was evidenced by the observation of the largest inhibition zone diameter at 27.6 mm. There was an apparent decrease in activity from day 3 to day 10, as demonstrated by the reduction in the diameter of the inhibition zone.

The *Streptomyces* sp. isolates demonstrated antibacterial activity against *Pseudomonas aeruginosa* ATCC 27853, with the effect becoming evident on day 2 and reaching its peak on day 7. The presence of the largest inhibition zone diameter at 24.3 mm was indicative of antibacterial activity. On day 8, a decrease in activity was observed, with the inhibition zone diameter reducing to 13.6 mm. Later, on day 9, the activity began to stop.

The *Streptomyces* sp. isolates showed antibacterial activity against *Escherichia coli* ATCC 25922, starting on day 1 and attaining maximum activity on day 5. The largest diameter of the inhibition zone measured was 29.2 mm. However, there was a decline in activity on day 6, as suggested by the decreasing diameter of the inhibition zone. On day 6, an inhibition zone with a diameter of 24.5 mm was observed. The inhibition zone diameter shrank to 21.8 mm on day 7 and 20.7 mm on day 8. By day 9, the inhibitory activity started to stop.

Lastly, the *Streptomyces* sp. isolates demonstrated antibacterial activity against *Salmonella typhimurium*, which was initially observed on day 2. The activity reached its peak on day 7, as indicated by the largest inhibition zone diameter of 27.5 mm. Nevertheless, there was a decrease in activity on day 8, as shown by the diminishing diameter of the inhibition zone. The activity came to a halt on day 10.

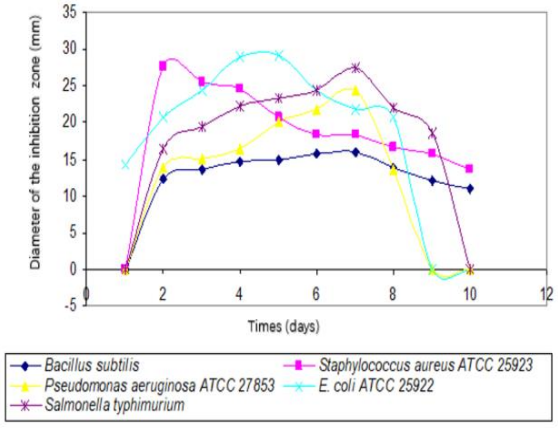

Figure 2. Activity profile of *Streptomyces* sp. according to the inhibition zones.

The antibacterial activity of *Streptomyces* sp. was expressed using inhibition zones, which were outlined throughout this section. Table 1 presents a more comprehensive set of data regarding the inhibition zones. Meanwhile, Figure 2 displays the activity profile of *Streptomyces* sp. in terms of its ability to inhibit the growth of the test bacteria.

Table 1. Inhibition zones from the antibacterial activity test of *Streptomyces* sp. using the modified agar diffusion method.

Groups	Repetition	Diameter of the inhibition zone around the test bacteria (mm)				
		B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*	*S. Typhimurium*
Positive c.	1st	21.5	14.3	16.3	12.5	11.8
	2nd	20.9	13.5	16.5	11.9	12.9
	3rd	21.8	14.2	15.8	11.6	12.8
	Mean	21.4	14	16.2	12	12.5
Negative c.	1st	0	0	0	0	0
	2nd	0	0	0	0	0
	3rd	0	0	0	0	0
	Mean	0	0	0	0	0
Day 1	1st	0	0	0	13.4	0
	2nd	0	0	0	14.4	0
	3rd	0	0	0	15.1	0
	Mean	0	0	0	14.3	0
Day 2	1st	12.3	27.1	0	21	16.7
	2nd	10.9	27.1	0	20.5	18
	3rd	13.4	28.6	0	20.9	14.8
	Mean	12.2	27.6	0	20.8	16.5
Day 3	1st	13	25.8	13	24.1	19.1
	2nd	13.5	26	13.6	25.2	19
	3rd	14.6	24.7	14.8	23.9	20.1
	Mean	13.7	25.5	13.8	24.4	19.4
Day 4	1st	15.3	25.3	16	28.6	22.3
	2nd	13.9	26	14.7	27.9	22.7
	3rd	14.9	22.8	14.9	30.2	21.9
	Mean	14.7	24.7	15.2	28.9	22.3
Day 5	1st	13.8	19.8	15.9	29.1	21.8
	2nd	15.8	21.1	16.4	20.1	23.8
	3rd	15.1	21.5	16.6	28.4	24.3
	Mean	14.9	20.8	16.3	29.2	23.3
Day 6	1st	16.4	18.5	20.5	23.9	23.9
	2nd	15.8	18	20.1	24.5	25.5
	3rd	15.2	18.4	19.4	25.1	24.1
	Mean	15.8	18.3	20	24.5	24.5
Day 7	1st	14.9	17.6	20.4	23.3	27
	2nd	15.5	19.1	22	21.8	28.3
	3rd	17.3	18.2	23.3	20.3	27.2
	Mean	15.9	18.3	21.9	21.8	27.5
Day 8	1st	13.5	17.7	24	18.8	21.4
	2nd	15	16.1	25.2	22.3	21.6
	3rd	13.2	16.3	23.7	21	23
	Mean	13.9	16.7	24.3	20.7	22
Day 9	1st	10.8	16.5	14.3	0	19.1
	2nd	12.5	14.9	15.5	0	18.3
	3rd	13	16	11	0	18.4
	Mean	12.1	15.8	13.6	0	18.6
Day 10	1st	10.1	14.1	0	0	0
	2nd	11.1	13	0	0	0
	3rd	11.8	13.4	0	0	0
	Mean	11	13.5	0	0	0

DISCUSSION

The findings of this study showed that *Streptomyces* sp. was able to produce antibiotics since day 1 of observation, as evidenced by the presence of a clear zone around *Escherichia coli* ATCC 25922. Furthermore, more antibiotics were produced on day 2, resulting in the inhibition of all the test microbes. Antibiotics produced by the *Streptomyces* sp. isolates apparently had varying inhibitory abilities against different test bacteria. The antibiotics exhibited the highest inhibitory power against *Escherichia coli* ATCC 25922 and the lowest inhibitory power against *Bacillus subtilis*. Regrettably, a gradual decline in antibiotic production was seen over time, which was generally notable on day 8. It even became ineffective in inhibiting the growth of *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 on day 9. Nevertheless, the qualitative test of antibacterial activity revealed that the antibiotic-producing *Streptomyces* sp. isolated from the Surabaya mangrove ecosystem has significant potential. The antibiotic potency of *Streptomyces* sp. was evident from its superior inhibitory power compared to the positive control of streptomycin at 250 ppm against the test bacteria. The considerable antibacterial activity of *Streptomyces* sp. isolates against pathogenic bacteria presents a promising opportunity for the development of a highly effective broad-spectrum antibiotic as an alternative treatment for infectious diseases (Balasubramanian et al. 2021). This recommendation aligns with the findings of previous studies on the antimicrobial activity of *Streptomyces* sp., specifically the AA13 and SA32 strains. *Streptomyces* sp. isolates found in the sediments of Lake Oubeira, Algeria, were found to have an inhibitory effect against *Candida albicans* (Adel et al. 2016, Ryandini et al. 2021).

The bacterial strains used for the experiments in this study were from the Gram-positive and Gram-negative classes. The Gram-positive bacteria were *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis*. Meanwhile, the Gram-negative bacteria were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella typhimurium*. The selection of the test bacteria was determined by taking into account their strong activity, clinical significance, and classification as Gram-positive and Gram-negative bacteria. These criteria allowed this study to assess the potency of broad-spectrum antibiotics. *Bacillus subtilis* is a bacterium with the shape of a rod and the ability to form spores. *Staphylococcus aureus* is a bacterium characterized by its spherical shape and ability to clinically invade the entire body (Tong et al. 2015).

Escherichia coli is a rod-shaped bacterium that can cause numerous diseases, such as urinary tract infections, pneumonia, meningitis, and septicemia. Similarly, both *Pseudomonas aeruginosa* and *Salmonella typhimurium* have a rod shape and can cause infectious diseases in humans. *Salmonella typhimurium*, in particular, is a major causative agent of gastroenteritis. Although many bacteria have shown resistance to certain antibacterials, there are still several effective options, such as the aminoglycoside group of antibiotics that includes amikacin and gentamicin (Percival & Williams 2014, Moradali et al. 2017).

The antimicrobial activity in this study was tested using the modified agar diffusion method. The diameter of the apparent inhibition zone was recorded, and all tests were performed in triplicate (Kumar et al. 2014, Palla et al. 2018). This method is a more advantageous alternative for the

assessment of the antimicrobial activity of *Streptomyces* sp. in comparison to the dilution method. This is due to the fact that *Streptomyces* sp., being a bacterium that shares resemblance to a fungus, can grow on both bacterial and fungal media. The dilution method is commonly employed to assess the inhibitory effects of the antibiotic produced by *Streptomyces* sp. against the growth of test bacteria. However, it is worth noting that the continuous cell division in *Streptomyces* sp. may result in turbidity, hence limiting the readability of the results obtained using the dilution method. In addition, the diffusion method is more preferable due to its ease of application and cost-effectiveness. The only drawback of this method is associated with the fluctuations in the growth of *Streptomyces* sp. in ISP-4 culture media. The quantity of *Streptomyces* sp. obtained from the mold may change in every experiment, hence complicating the quantitative measurement of the inhibition zone diameter. Consequently, plenty of replications are required to address this issue.

This study used standard streptomycin sulfate, which is classified as an aminoglycoside derivative, to determine the responsiveness of the test bacteria towards the antibiotic and the suitability of these test bacteria for experimentation (Martineza et al. 2014). The initial step in the antibacterial activity test was the fermentation process of the *Streptomyces* sp. antibiotic. In the process of testing antibacterial activity, nutrient agar was used as a non-selective medium to facilitate the growth of the microbes. The thickness of the media, which contained the test microbes, must remain consistent and homogeneous to avoid fluctuations in the diameter of the resulting inhibition zones. The selected test microbes should have a transmittance level of 25% (Fathoni et al. 2021). This particular transmittance level ensures that the number of bacterial cells is appropriate for the test and fits within the logarithmic growth phase. This condition maximizes the sensitivity of the microbes to antibiotics. The test results of this study revealed variations in the antibacterial activities of *Streptomyces* sp. isolates against the test bacteria in terms of their ability to induce inhibition, the activity duration, and the inhibition zone size. These findings suggested that the secondary metabolites produced by the *Streptomyces* sp. isolates had varying degrees of strength against *Bacillus subtilis*, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Salmonella typhimurium*.

Strength and limitations

This study can contribute to the development of antibiotic products by isolating *Streptomyces* sp. from highly polluted environments. By doing so, this study attempted to obtain robust and specific isolates that can survive in challenging environments, produce antibiotics, and offer sources of biodiversity that have not been widely disclosed. However, only macroscopic and microscopic observations were conducted for the identification of *Streptomyces* sp. and the assessment of its antibacterial activity. Further research at the molecular level is necessary to ascertain the presence of *Streptomyces* sp. in the soil of mangrove ecosystems.

CONCLUSION

Streptomyces sp. derived from mangrove ecosystem soil has inhibitory effects against Gram-positive and Gram-negative bacteria. This species produces secondary metabolites that possess significant potential. These metabolites offer promising opportunities to be developed as alternative drugs for infectious diseases by providing enhanced effectiveness as broad-spectrum antibiotics.

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Conflict of interest

None.

Ethical consideration

This study received an ethical exemption from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, with certificate No. 176/EC/KEPK/FKUA/2023 on 10/7/2023.

Funding disclosure

None.

Author contribution

WR contributed to the conception and design, collection and assembly of the data, analysis and interpretation of the data, drafting of the article, and critical revision of important intellectual content. NMM contributed to the conception and design, analysis and interpretation of the data, drafting of the article, and critical revision of important intellectual content. MP and NW contributed to the administrative, technical, and logistic support as well as the drafting of the article and the critical revision of important intellectual content. A contributed to the critical revision of important

intellectual content. SM contributed to the administrative, technical, and logistic support. WM contributed to the drafting of the article and the critical revision of important intellectual content.

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Original Research Report

AURICULAR ACUPRESSURE EFFECTIVELY REDUCES LABOR ANXIETY IN PRIMIGRAVIDAE BY INCREASING β-ENDORPHIN LEVELS

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ABSTRACT

Anxiety is a common psychological symptom in expectant mothers, particularly among primigravidae during labor. There is a link between excessive anxiety and prolonged labor. One of the options to manage anxiety is the use of non-pharmacological methods. Numerous studies have investigated the effectiveness of auricular acupressure in treating anxiety, but few have examined its effect on anxiety biomarkers. Thus, the objective of this study was to analyze the effect of auricular acupressure targeting the Shenmen point on the levels of β-endorphins and anxiety among primigravidae during the first stage of labor. This experimental study used a pretest-posttest control group design. The research subjects were primigravidae in the first stage of labor who were examined at the independent midwife practice of Yefi Marliandiani in Surabaya, Indonesia. Among 40 primigravidae, 34 individuals met the inclusion criteria and were then equally divided into treatment and control groups using simple randomization. The treatment group received auricular acupressure at the Shenmen point every 30 minutes for 5 minutes each time, while the control group practiced deep breathing relaxation. The anxiety levels were assessed using the Hamilton Anxiety Rating Scale. The β-endorphin levels were measured by drawing 3 cc of venous blood before (4 cm cervix dilation) and after (9–10 cm cervix dilation) treatment. The obtained data were analyzed using paired-sample and independent-sample t-tests. A decrease in the mean anxiety level was observed in the treatment group (18.59±7.52) relative to the control group (19.76±7.11), albeit not significant (p=0.64). Simultaneously, the average β-endorphin levels of the treatment group (355.4±224.7) increased compared to the control group (225.1±127.5), with a significant difference observed between the two groups (p=0.04). In conclusion, while both auricular acupressure at the Shenmen point and deep breathing relaxation are effective in reducing anxiety, the former is more prominent in increasing β-endorphin levels.

Keywords: Childbirth complications; auricular acupressure; primigravidae; anxiety; β-endorphins

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Highlights:
1. Auricular acupressure at the Shenmen point is effective in reducing anxiety.
2. Auricular acupressure is more practical than body acupressure since it does not interfere with expectant mothers' mobilization.
3. As a non-pharmacological method, auricular acupressure is cost-effective, efficient, and effective in overcoming maternal anxiety during labor.

INTRODUCTION

Expectant mothers frequently experience anxiety throughout the labor process. Pregnant women may have feelings of anxiety as a result of insufficient knowledge or information regarding pregnancy and childbirth. This phenomenon is particularly true for women who are pregnant for the first time, also known as primigravidae. Previous research findings showed that providing prenatal education and counseling regarding the physiological changes during pregnancy and childbirth can reduce stress and anxiety levels, consequently minimizing the probability of interventions during the labor process (Firouzbakht et al. 2015).

Anxiety is an emotional state characterized by a combination of worry and fear. According to the principles of Traditional Chinese Medicine (TCM), anxiety arises from an insufficiency of energy that produces cold (*xue* or *yin*), an imbalance due to excessive heat energy (*yang*), or a combination of both (Mafetoni et al. 2018). The Shenmen point is purported to possess the capacity to modulate numerous therapeutic outcomes. Auricular acupressure targeting the Shenment point can calm the mind, reduce stress, and manage sleep disorders, pain, and withdrawal symptoms (Tseng et al. 2015).

The presence of anxiety among pregnant women has the potential to prolong the labor process. There is an association between prolonged labor and maternal mortality in Indonesia. The findings from an analysis of the 2017 Indonesian Demographic and Health Survey (IDHS) revealed a 44.9% prevalence rate of prolonged labor (Annisya 2020).

The main factors associated with prolonged labor in primigravidae are referred to as the 5P, which includes powers, passenger, passage, psychology, and partner. Prior research reported a significant relationship (p=0.000) between uterine contractions and the occurrence of prolonged labor (Putri 2019). The feeling of anxiety throughout the labor process may lead to irregular uterine contractions and affect the psychology of the mother. Another study revealed a statistically significant relationship (p=0.009) between anxiety and the duration of labor (Sagita 2018).

Maternal anxiety causes a spike in the hormone catecholamines, consequently disrupting uterine contractions. Physiological labor contractions are initiated by a decrease in progesterone levels and an increase in prostaglandin levels. Prostaglandin serves as a receptor for oxytocin, and suboptimal synthesis of this hormone can lead to uterine inertia, which can interfere with or prolong the birth process (Caughey et al. 2014, Bauer 2019).

Heightened anxiety levels in women during the labor process have been found to result in increased stress. A condition of stress induces tension inside the body, particularly in the muscles of the birth canal. This tension manifests as muscle stiffness and hardness, obstructing cervix dilation and consequently disrupting the progression of labor (Sagita 2018). Maternal anxiety may arise from an individual's response to pain or anticipation of what is to come during labor. The management of anxiety for women nearing labor at primary health services includes prenatal yoga, deep breathing relaxation, aromatherapy, acupressure, and acupuncture. However, as of the conduct of this study, there has been a scarcity of empirical research examining biomarkers associated with the effectiveness of non-pharmacological methods for anxiety management in childbirth.

One of the recommended preventive measures for maternal anxiety is counseling and non-pharmacological methods such as auricular acupuncture. The purpose of applying acupressure to the auricle, or external ear, is to stimulate the nerves inside this region that are directly connected to the brain. Several major nerve branches in the external ear include the major auricular nerve, minor occipital nerve, trigeminal nerve, temporal auricular nerve, facial nerve, vagus nerve, and glossopharyngeal nerve. These nerves are part of the sympathetic, parasympathetic, and autonomic nervous systems, which are associated with the brain, brain stem, and cerebral cortex (Saputra & Sudirman 2009, Kaniusas et al. 2019).

The effectiveness of auricular point stimulation in anxiety reduction has been assessed in multiple studies. The application of auriculotherapy to expectant mothers was found to be associated with reduced levels of anxiety in comparison to the control groups (RR 4.88 CI95% 1.87-7.88, ρ=0.0015). The research used the Hamilton Anxiety Rating Scale as an instrument for assessing the anxiety levels of pregnant women (Mafetoni et al. 2018). Another study also demonstrated a similar result in terms of the beneficial effects of auriculotherapy. The effectiveness of this therapeutic intervention in reducing anxiety was demonstrated in the experimental group (ρ=0.001) (Vakilian et al. 2022). However, the lack of empirical evidence and research on the use of auriculotherapy in primigravidae during labor restricts the extent of knowledge and practical implementation of auricular acupressure therapy in the field of midwifery. Hence, the aim of this study was to analyze the effect of auricular acupressure, specifically targeting the Shenmen acupoint, on the anxiety levels of primigravidae during labor by assessing the interleukin-6 biomarker.

MATERIALS AND METHODS

The design of this study was true experimental, particularly the pretest-posttest control group design. This study was conducted from April to September 2022, involving a population of 40 primigravidae at the independent midwife practice of Yefi Marliandiani in Surabaya, Indonesia. A total of 34 primigravidae who met the inclusion criteria were included as the samples in this study. The inclusion criteria comprised a gestational age of 37–40 weeks, a low-risk pregnancy, a Poedji Rochjati score of 2–6, the absence of any skin disease on the ears, and progression into the latent phase of the first stage of labor (Prakasiwi et al. 2023). This study

excluded primigravidae who had complicated births, emergency deliveries, inadequate antenatal care, or less than four antenatal visits. The subjects that met the inclusion criteria were randomly assigned to groups using a random number table, which was prepared by a statistician (Egbewale 2014). The subjects were divided into two distinct groups: the treatment group and the control group. The treatment group received auricular acupressure at the Shenmen point, while the control group received instructions to practice deep breathing relaxation. This study used a double-blind method, wherein both the statisticians and analysts were blind to the distribution of research subjects to either the treatment or control group.

The anxiety levels of primigravidae were assessed twice using the Hamilton Anxiety Rating Scale (HARS), i.e., before and after a 120-minute treatment session. The scores given to each item on the Hamilton Anxiety Rating Scale were as follows: none=0, mild=1, moderate=2, severe=3, and very severe=4. The total score ranged from 0 to 56. The scores were categorized into the following classifications: no anxiety (<6), mild anxiety (6–14), moderate anxiety (15–27), severe anxiety (28–41), and very severe anxiety (42–56) (Nursalam 2020).

Medical analysts collected 3 cc of venous blood samples for the examination of β-endorphin levels. The collection of blood samples was conducted on two occasions, prior to and following treatment, when the cervix dilated to 9–10 cm. The blood serum was subsequently transported to the GAKI Laboratory of the Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia, where it was stored in a deep freezer at a temperature of -80°C. The enzyme-linked immunosorbent assay (ELISA) kit was used to test the blood serum samples for β-endorphin levels after all samples had been collected (Clark & Engvall 2018).

The experiment group was subjected to auricular acupressure at the Shenmen point, as shown in Figure 1. The shenmen acupoint is located in the triangular fossa and is innervated by the vagus and trigeminal nerves. These nerves have a role in influencing the brainstem and cortex to receive, condition, and encode auricular reflexes with sedative and analgesic effects. The auricular acupressure was performed by the researchers, who had attended six months of medical acupuncture training at the Acupuncture Research Laboratory in Health Services (LP3A) in Surabaya, Indonesia. Stimulation was applied every 30 minutes for a duration of 5 minutes in a clockwise manner to induce *deqi* (Setiawandari et al. 2022). The term "*deqi*" denotes the sensations that arise with the application of acupuncture at a certain acupoint. It refers to the sensations felt by the patient as well as the sensory perceptions identified by the acupuncturist.

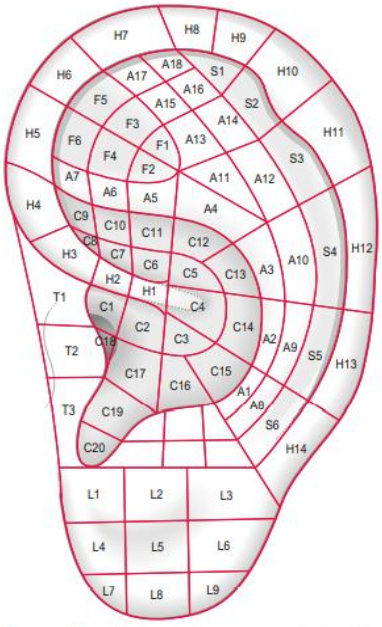

Figure 1. The auricular acupressure nomenclature from the report of the World Health Organization working group (Romoli 2009).

The control group received standard midwifery care for anxiety management, which comprised deep breathing relaxation techniques. A midwife who had completed the Normal Childbirth Care and Midwifery Update Training assisted the subjects in practicing deep breathing relaxation. The progress of labor was observed in accordance with the partograph developed by the World Health Organization (Hofmeyr et al. 2021). The data were analyzed using paired- and independent-samples t-tests ($p<0.05$) in IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, N.Y., USA). This study received approval from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia, with protocol No. 352/EC/KEPK/FK-UNDIP/IX/2021 on 2/9/2021.

RESULTS

From the total population of 40 expectant mothers in the labor process, 34 individuals participated as research subjects in this study. The 34 primigrividae were divided into two groups, i.e., the treatment group that received auricular acupressure and the control group that received instruction to practice deep breathing relaxation. The primigravidae in the first stage of labor were assessed for their anxiety levels using the Hamilton Anxiety Rating Scale before and after applying the 120-minute treatment

session. Figure 2 shows the Consolidated Standards of Reporting Trials (CONSORT) diagram that demonstrates the research flow.

Table 1 shows the demographic characteristics of the research subjects involved in this study. In comparison to the treatment group, the research subjects in the control group had a higher mean age of 23.6 years (SD=3.01). The treatment group consisted of individuals with a mean age of 22.0 years (SD=2.58). However, the statistical analysis revealed that there was no significant difference in the age distribution among the research subjects (p=0.4). The majority of the research subjects were high school graduates, with 88.2% in the treatment group and 70.6% in the control group. There was no statistically significant difference observed in the distribution of education levels between the two groups (p=0.5). Most of the research subjects were housewives, with 70.6% in the treatment group and 58.8% in the control group. The statistical analysis showed that there was no significant difference in the occupational distribution of the research subjects (p=0.3). The control group exhibited a marginally higher mean gestational age of 38.8 months (SD=0.98) compared to the treatment group's average of 37.6 months (SD=0.97). The statistical analysis indicated that there was no significant difference observed between the two groups (p=0.5).

Table 1. Demographic characteristics of the research subjects.

Characteristics	Groups		p
	Treatment (n=17)	Control (n=17)	
Age			0.4*
Mean±SD	22.0±2.58	23.6±3.01	
Median (min-max)	24.5 (18-29)	24.5 (18-29)	
Education			0.5**
Middle school	1 (5.9%)	1 (5.9%)	
High school	15 (88.2%)	12 (70.6%)	
Diploma	1 (5.9%)	4 (23.5%)	
Occupation			0.3**
Housewife	12 (70.6%)	10 (58.8%)	
Self-employed	2 (11.8%)	5 (11.8%)	
Private employee	3 (17.6%)	2 (29.4%)	
Gestational age			0.5***
Mean±SD	37.6±0.97	35.4±0.86	
Median (min-max)	39.0 (37-40)	39.0 (37-40)	

Notes: *Unpaired t-test; **Fisher's exact test; ***Mann-Whitney test, significance (p<0.05).

Table 2 presents the prevalence of anxiety among the research subjects in both the treatment and control groups. The severity of anxiety was measured in accordance with the classification of the Hamilton Anxiety Rating Scale. Prior to the intervention, severe anxiety was the most prevalent condition among the primigravidae in the treatment group (41%). Similarly, severe anxiety emerged as the most predominant condition within the control group (53%). Following the intervention, it was seen that mild anxiety became the most prevalent category among research subjects in the treatment group (47%) of the participants. In contrast, the control group continued to exhibit a prominent presence of severe anxiety (41%).

Table 2. Anxiety prevalence among the research subjects according to the HARS scores.

Scores	Pretest		Posttest	
	Treatment (n=17)	Control (n=17)	Treatment (n=17)	Control (n=17)
<6 (no anxiety)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
6–14 (mild anxiety)	4 (24%)	2 (12%)	8 (47%)	5 (29%)
15–27 (moderate anxiety)	6 (35%)	6 (35%)	5 (29%)	5 (29%)
28–41 (severe anxiety)	7 (41%)	9 (53%)	4 (24%)	7 (41%)
42–56 (very severe anxiety)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
p*	0.26	0.45	0.16	0.36

Notes: *significant p-value of the Shapiro-Wilk test (p<0.05).
HARS: The Hamilton Anxiety Rating Scale.

According to the data presented in Table 3, a significant decrease (p=0.000) was observed in the measurements taken before and after the application of auricular acupressure and deep breathing relaxation techniques. Therefore, it was implied that the use of auricular acupressure and deep breathing relaxation had an impact on the levels of anxiety experienced by primigravidae during the first stage of labor. The treatment group exhibited lower levels of anxiety in comparison to the control group, as indicated by the mean scores obtained from the measurement using the Hamilton Anxiety Rating Scale. The average score in the treatment group was 18.59 (SD=7.52), while it was 19.76 (SD=7.11) in the control group. Nevertheless, the statistical analysis revealed that the observed difference was not significant (p=0.64).

The N-gain score observed in the treatment group was -32.8%, indicating that the use of the auricular acupressure technique did not significantly reduce anxiety levels among primigravidae. On the other hand, the control group exhibited an N-gain score of 65.8%, suggesting that the deep breathing relaxation technique was fairly effective in reducing anxiety levels among primigrividae. This study found a difference in the comparison of effectiveness between auricular acupressure and deep breathing relaxation techniques, albeit without statistical significance (p=0.16).

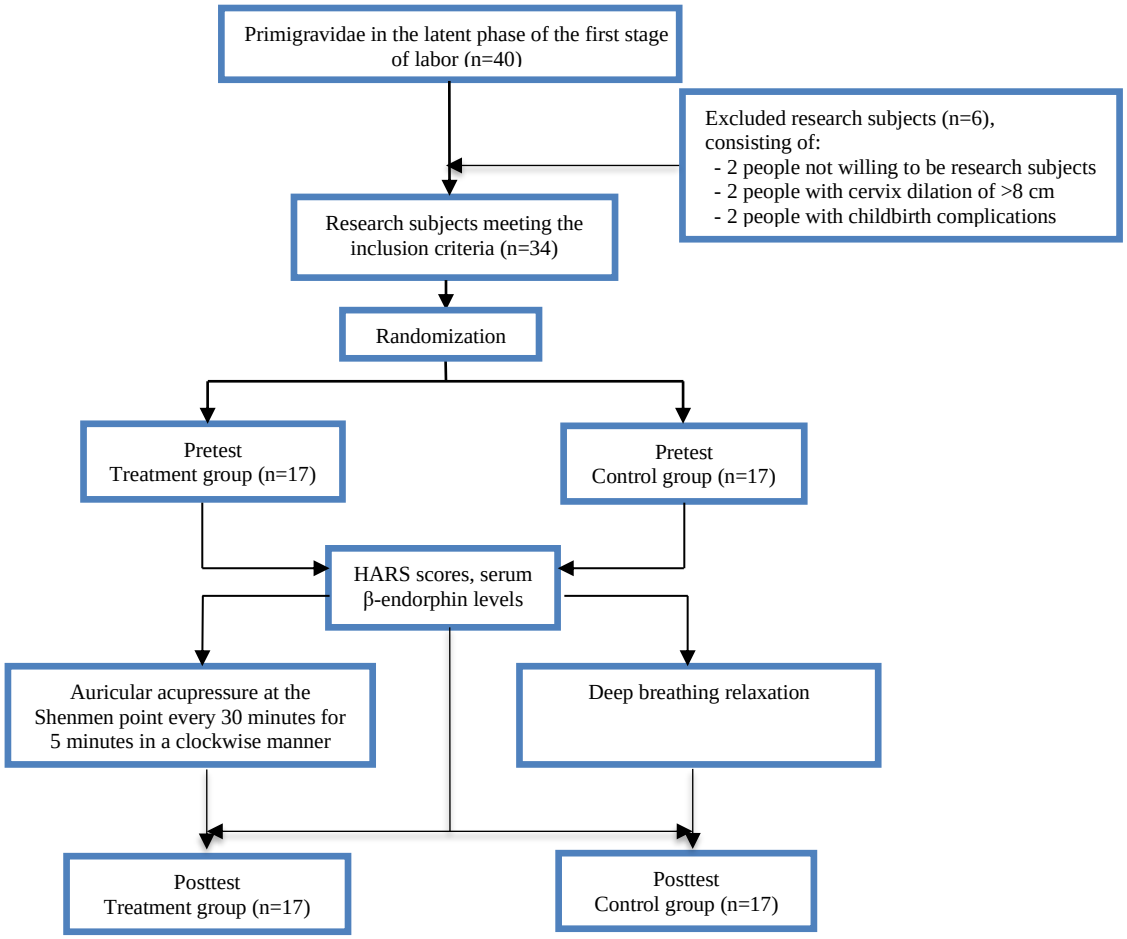

Figure 2. Consolidated Standards of Reporting Trials (CONSORT) diagram of the study population and research flow.

Table 3. Pretest and posttest anxiety levels in the treatment and control groups.

Anxiety levels (HARS scores)	Groups		p
	Treatment (n=17)	Control (n=17)	
Pretest			
Mean±SD	22.18±7.00	24.59±6.74	
Median (min-max)	22 (12-34)	21 (12-33)	
Posttest			0.64**
Mean±SD	18.59±7.52	19.76±7.11	
Median (min-max)	20 (9-29)	20 (10-30)	
N-gain percentage			0.16**
Mean±SD	-32.8±148.1	65.8±237.3	
Median (min-max)	-21.4 (-450–300)	25 (-400–600)	
p	0.000*	0.000*	

Notes: *paired sample t-test; **independent sample t-test, significance (p<0.05).

Table 4 presents the categorization of pre- and post-treatment serum β-endorphin assessment results for both groups. Prior to the therapeutic intervention, it was observed that 53% of the research subjects in the treatment group had abnormal β-endorphin levels. A similar situation was observed in 59% of the research subjects in the control group. Following the intervention, the majority of the research subjects in both treatment and control groups exhibited β-endorphin levels within the normal range, at 76% and 65%, respectively.

Table 4. Categorization of serum β-endorphin levels in the treatment and control groups.

β-endorphin levels	Pretest		Posttest	
	Treatment (n=17)	Control (n=17)	Treatment (n=17)	Control (n=17)
Abnormal (<202 pg/ml)	9 (53%)	7 (41%)	4 (24%)	6 (35%)
Normal (202–389 pg/ml)	8 (47%)	10 (59%)	13 (76%)	11 (65%)
p*	0.02	0.06	0.08	0.07

Notes: *paired sample t-test; **independent sample t-test, significance (p<0.05).

As shown in Table 5, the pre-treatment serum β-endorphin level in the treatment group was higher at 298.6 pg/mL (SD=211.6) compared to the control group's β-endorphin level of 223.4 pg/mL (SD=176.3). Following the completion of the treatment, it was found that the treatment group maintained a higher serum β-endorphin level of 355.4 pg/mL (SD=224.7) than the control group, which had a β-endorphin level of 225.1 pg/mL (SD=127.5). The statistical analysis revealed a significant difference between the two groups (p=0.04).

Table 5. Pretest and posttest serum β-endorphin levels in the treatment and control groups.

β-endorphin levels	Groups		p**
	Treatment (n=17)	Control (n=17)	
Pretest			
Mean±SD	298.6±211.6	223.4±176.3	
Median	277	185	
(min-max)	(78-835)	(54-714)	0.04
Posttest			
Mean±SD	355.4±224.7	225.1±127.5	
Median	287	227	
(min-max)	(87-913)	(61-443)	
N-gain percentage			0.2
Mean±SD	-134±535.9	23.8±74.8	
Median	2.89	12.23	
(min-max)	(-216–171.1)	(-66.7–270)	
p* (pre vs. post)	0.006	0.9	

Notes: *paired sample t-test; **independent sample t-test, significance (p<0.05).

In the treatment group, there was a significant difference (p=0.006) in the serum β-endorphin levels before and after the research subjects received auricular acupressure. This indicated that auricular acupressure at the Shenmen point has an effect on serum β-endorphin levels. Conversely, in the control group, there was no statistically significant difference in serum β-endorphin levels before and after the use of the deep breathing relaxation technique (p = 0.9). However, the calculation of N-gain scores revealed no statistically significant difference (p=0.2) in the effectiveness of auricular acupressure and deep breathing techniques for increasing serum β-endorphin levels in primigravidae during the first stage of labor.

DISCUSSION

The primigravidae in the labor process that participated in this study had similar characteristics in terms of age, education, occupation, and gestational age, with no statistically significant differences between the treatment and control groups. The average age of the mothers ranged from 22 to 23 years, with the youngest mother being 18 years old and the oldest mother being 29 years old. The findings from previous studies indicate that age has a significant role in influencing maternal anxiety levels during childbirth. Maternal age even had the greatest influence on stress levels during pregnancy, according to a study by Ayu et al. (2019). In the study, it was found that teenage mothers had a 10.31 times higher chance of experiencing prenatal stress compared to adult mothers. The lower the mother's age, the more psychosocial problems there will be. Conversely, an increase in the mother's age is associated with more social support and a higher ability to adjust to changes throughout pregnancy, leading to a greater sense of relaxation.

Most primigrividae in this study had completed a high school education, indicating that they had sufficient knowledge regarding pregnancy and childbirth. The maternal education level allowed the mothers to receive information from midwives or social media. Mothers who had not received prenatal care or had fewer than four antenatal visits were excluded from this study to avoid any bias related to the information acquired by mothers during antenatal care. According to a recent study conducted by Çankaya & Şimşek (2021), it was found that pregnant women who received antenatal education had lower levels of depression, anxiety, fear of childbirth, and stress symptoms compared to the control group. Additionally, these women demonstrated increased childbirth self-efficacy, resulting in a higher occurrence of vaginal births (p=0.043). The provision of childbirth education has been found to have a significant effect on reducing maternal anxiety levels. Sufficient knowledge regarding childbirth is associated with smooth labor and delivery, as well as a decreased inclination among expectant women to choose the cesarean section (Ritonga & Hidayat 2023).

According to this study, before the implementation of the therapeutic intervention, the majority of the mothers in both the treatment group (41%) and the control group (53%) experienced severe anxiety. The treatment group had an average level of anxiety that was 2.41 times lower compared to the control group. Following the use of auricular acupressure, it was observed that mild anxiety emerged as the most predominant condition within the treatment group (47%). Anxiety is a common problem faced by women during childbirth, particularly primi-gravidae. The lack of information regarding pregnancy, unfamiliar circumstances, and limited understanding of childbirth among primigravidae, coupled with the anticipation of potential interventions, contribute to heightened levels of stress and anxiety (Mafetoni et al. 2018).

Severe anxiety towards childbirth greatly influences the duration of labor in primigravidae. On the contrary, it has been observed that multigravidae,

who have carried several pregnancies, tend to have a comparatively smoother childbirth due to lower levels of anxiety (Aral et al. 2014, Feng et al. 2017). A study by Shodiqoh & Syahrul (2014) found that primigravidae had a higher level of anxiety compared to multigravidae (p=0.006). Anxiety during childbirth can induce both physiological and psychological stress, hence negatively impacting the overall birthing process. Anxiety has been shown to prolong labor, which is correlated with reduced concentrations of oxytocin in the blood plasma. A positive relationship exists between the duration of labor and the concentration of β-endorphin, an endogenous opioid that is released in response to stress. The inhibition and regulation of oxytocin secretion via opioid-induced mechanisms play a crucial role in controlling labor contractions and preventing pathological uterine contractility.

The presence of stress in pregnant women can increase the opioid-mediated inhibition of oxytocin secretion. The inhibition potentially leads to a decrease in uterine contractility, hence obstructing the progress of labor. Furthermore, the concentrations of corticotrophin-releasing hormone (CRH) and β-endorphin in the maternal plasma may increase as a response to pain perception and increased stress during labor (Dixon et al. 2013, Walter et al. 2021). In the context of labor, situations that compromise women's sense of privacy, safety, and tranquility can trigger an increase in the levels of epinephrine and norepinephrine, which may slow or delay the labor process and reduce the fetal blood supply. Stress hormones, such as adrenaline, interact with receptors located in the uterine muscles to reduce their contractility (Buckley 2015, Ayu et al. 2019).

Efforts aimed at mitigating anxiety in expectant mothers include the provision of non-pharmacological interventions, such as the auricular acupressure technique. Auricular acupressure is a complementary therapy that applies pressure to the ear, which contains nerve fibers that connect to the entire human body. The objective of this therapy is to overcome energy imbalances, such as anxiety experienced by women during labor. The results of this study are in line with prior research, which showed that mothers who received auriculotherapy during labor had lower levels of anxiety, as indicated by the Hamilton Anxiety Rating Scale scores, in comparison to women in the other groups (Mafetoni et al. 2018).

A study by Novianti & Muchtar (2021) revealed that acupressure at the Yintang and Shenmen points produced a statistically significant effect (p=0.001) in reducing anxiety levels among primigravidae in labor. Another study showed comparable results regarding the beneficial effect of acupressure. The application of auricular acupressure at the Shenmen point was found to reduce anxiety and fatigue in mothers who had undergone a caesarean section compared to those who received standard care (Kuo et al. 2016). The Shenmen acupoint is known for its ability to calm the mind and soul, reduce anxiety, and produce analgesia and sedation. The study conducted by Silva et al. (2020) revealed that auriculotherapy significantly decreased anxiety levels (p=0.033). The effect was evident between the third and fourth applications of auriculotherapy.

The Shenmen point is located in the triangular fossa, an anatomical region innervated by the trigeminal and vagus nerves. Acupressure manipulation at the Shenmen point induces heightened neural activity, resulting in the transmission of impulses to the central nervous system. The central nervous system is responsible for stimulating the activation of the pituitary and endocrine systems. The secretion of the hormone β-endorphin by the pituitary gland, along with the modulation of the autonomic nervous system, can produce analgesic effects such as reduced pain perception (Dixon et al. 2013). This can be obtained by various mechanisms, such as raising the parasympathetic nervous response, decreasing the sympathetic nervous response, and reducing impulses in the nervous system.

No prior studies have established a correlation between auricular acupressure and the biomarkers associated with anxiety during labor. Senudin (2019) conducted a study by applying the acupressure technique at the EX-HN 3 and HT 7 points on pregnant women in their third trimester. The study demonstrated that the acupressure yielded a significant decrease in anxiety levels. However, it did not result in a significant increase in endorphin levels. Therefore, this present study further examined the effects of auricular acupressure specifically targeting the Shenmen point on the levels of β-endorphin, which indicated the anxiety levels of primigravidae during labor.

The results of this study revealed a statistically significant difference (p=0.04) in serum β-endorphin levels between the treatment and control groups following the intervention. The treatment group exhibited higher levels of serum β-endorphin, with a mean increase of 56.8 pg/mL (ρ=0.006). In prior research, it was observed that there was an increase in the levels of β-endorphin in both the treatment and control groups. However, the treatment group had a significantly higher increase in β-endorphin levels at 88.57 ± 52.46 pg/mL in comparison to the control group's increase of 12.86 ± 56.76 pg/mL (p=0.001) (Saraswati et al. 2021). In a separate study, the application of auricular acupressure at the Shenmen point was found to result in decreased cortisol levels. The average

difference between the treatment and control groups was measured at 4 µg/dL. Consequently, auricular acupressure was proven to reduce anxiety and fatigue symptoms on day 5 postpartum (Kuo et al. 2016).

The effects of acupressure manifest at various levels within the nervous system, including the peripheral, segmental (spinal), and central levels. Numerous regions within the brain and spinal cord have been recognized as sites of action for endogenous opioids. These regions include the hypothalamus, limbic system, basal ganglia, periaqueductal gray area, raphe magnus nucleus, reticular activating system, and dorsal horn of the spinal cord. The Shenmen acupoint is known to have direct contact with the central nervous system, bypassing the body's meridian system. Thus, the Shenmen point plays an important role in reducing anxiety at a faster speed. This study revealed that auricular acupressure at the Shenmen point resulted in increased β-endorphin levels, hence providing a sense of comfort and relaxation for anxious expectant mothers. On the other hand, heightened maternal stress during labor may cause an excessive release of β-endorphins. Supraphysiological β-endorphin levels can inhibit the function of oxytocin, which subsequently slows down the progress of labor. According to the gate control theory, the application of auricular acupressure activates larger afferent fibers that can inhibit the transmission of pain and anxiety signals via smaller fibers. This mechanism provides a means to modulate the perception of labor pain and anxiety (Bonapace et al. 2018).

Strength and limitations

The findings of this study suggest that auricular acupressure has the potential to increase the secretion of β-endorphins. Hence, auricular acupressure can be useful as a complementary therapy to reduce the anxiety levels of pregnant women, particularly those in the labor process. Auricular acupressure may alleviate expectant mothers' anxiety through stimulation of the hypothalamus and anterior pituitary, resulting in increased production of β-endorphin. However, this study was limited to pregnant women undergoing natural childbirth, which imposed a time constraint on the research. The effect of auricular acupressure on prolonged or induced labor remains inconclusive, indicating the need for further research.

CONCLUSION

Both auricular acupressure at the Shenmen point and deep breathing relaxation techniques have comparable effectiveness in reducing anxiety levels among primigravidae. However, auricular acupressure targeting the Shenmen point is more effective in increasing β-endorphin levels compared to the deep breathing relaxation technique.

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Conflict of interest

None.

Ethical consideration

The Health Research Ethics Committee of the Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia, issued the ethical approval for this study with protocol No. 352/EC/KEPK/FK-UNDIP/IX/2021 on 2/9/2021.

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None.

Author contribution

S and KS contributed to the conceptualization, preliminary study, and provision of study materials. S and YKW contributed to the analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, and final approval of the article. SA contributed to the statistical expertise, the acquisition of funding, the provision of administrative, technical, and logistic support, and the collection and assembly of data.

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Original Research Report

PHYSICAL ACTIVITY LEVELS AND TOTAL FOOD INTAKE AMONG PRECLINICAL STUDENTS AT A SCHOOL OF MEDICINE AND HEALTH SCIENCES

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ABSTRACT

One of the most crucial ways a person can do to enhance the well-being of their body is to engage in regular physical activities. However, currently, many people still have low physical activity levels. It is stated that habitual physical activity can affect the sensitivity of food intake regulation. This study aimed to determine the correlation between the total food intake and physical activity of preclinical students. This study was conducted using the cross-sectional method among preclinical students (n=238) in the classes of 2017, 2018, and 2019 at the School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia. Subjects with appetite-manipulating supplements, a diet program, a history of metabolic disease, a history of eating disorders, stress, or trauma were excluded from this study. The physical activity level was evaluated using the Baecke Physical Activity Questionnaire (BPAQ), while the total food intake was evaluated using the three-day food record method. The statistical test applied in this study was the Spearman test, with a significance of $p<0.05$. This study found that 22.3% of the respondents had a low physical activity level, and 9.7% of the respondents had a total food consumption higher than the recommended intake. The statistical analysis showed a significant negative correlation between physical activity levels and total food intake ($p=0.008$). In conclusion, there is an association between students' physical activity levels and their total food intake.

Keywords: Physical activity; total food intake; health risk

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Highlights:
1. This is the first cross-sectional study in Indonesia that was conducted specifically to find a correlation between physical activity levels and food intake.
2. As lower physical activity was found to be associated with higher food intake, this study suggests that physical activity might be taken into consideration to manipulate one's food consumption.

INTRODUCTION

Body weight is strongly influenced by energy balance, which consists of caloric intake and energy expenditure. Caloric or food intake that exceeds energy expenditure will create an energy surplus, increase body weight, and lead to obesity. Conversely, food intake that is less than energy expenditure will cause an energy deficit and reduce a person's weight (Romieu et al. 2017). In Indonesia, the prevalence of obesity among adults was approximately 14.8% in 2013. The number increased to 21.8% in 2018, according to basic health research in the country (Minister of Health of the Republic of Indonesia 2013, 2018). A study by Poobalan & Aucott (2016) found an increase in

obesity and overweight among young adults. The increased prevalence of obesity was most evident among students. These findings match those of another study conducted by Peltzer et al. (2014) that discovered a relationship between obesity, overweight, and inactivity.

Everyone has varying daily calorie requirements based on their age and gender. The consumption of more food than required is known as overconsumption (Finlayson 2017, Minister of Health of the Republic of Indonesia 2019). There have been limited studies that have particularly investigated the prevalence of overconsumption in Indonesia. According to the American Psychological Association (2013), about 83% of adults in the United States overeat. Excessive food intake can have a variety of consequences. People who overeat may experience body fat accumulation, altered appetite regulation, and an increased risk of various illnesses (Johnson & Wardle 2014, Leaf & Antonio 2017, Cercato & Fonseca 2019).

A person's total food intake is influenced by appetite regulation, which is affected by several factors that can be divided into internal and external factors. The internal factors consist of a history of genetic disorders, stress, and certain diseases (Grimm & Steinle 2011, Morton et al. 2014, Batchelor & German 2019), while the external factors consist of a history of trauma, the consumption of drugs, and the consumption of supplements to increase or reduce appetite. Habitual physical activity can enhance appetite regulation, according to research on exercise levels and appetite control by Morton et al. (2014) and Beaulieu et al. (2016). It was also found that there was an increase in short-term control of food intake among subjects who exercised regularly compared to those who were inactive. Several studies indicate that exercise training reduces meal size while eating a calorie-dense meal but not when eating a low-calorie meal. People who engage in less physical exercise show a preference for calorie-dense meals. This phenomenon occurs due to the influence of physical activity on insulin resistance, thus affecting hunger management (Beaulieu et al. 2016).

The purpose of this study was to determine the correlation between overall dietary consumption and physical activity among preclinical students enrolled in the School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia. To the best of the authors' knowledge, this study represents the first cross-sectional investigation conducted in Indonesia that examined the correlation between physical activity levels and food intake.

MATERIALS AND METHODS

The subjects of this study were preclinical students in the classes of 2017, 2018, and 2019 at the School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia. A total of 238 preclinical students, 66 males and 172 females, were included in the study by using a simple random sampling method. The exclusion criteria were those who consumed supplements to enhance or reduce appetite, were on a diet program, had a history of metabolic disorders (e.g., diabetes mellitus, hyperthyroidism, or hypothyroidism), had a history of bulimia or anorexia nervosa, and were stressed or traumatized. The subjects were requested to complete an online survey using Google Forms, providing personal details such as name, age, sex, and anthropometric information, including height and weight. Physical activity levels were assessed using the Baecke Physical Activity Questionnaire (BPAQ) (Oyeyemi et al. 2016). The categorization of physical activity levels (PAL) used in this study was designed for the average adult population. These levels were categorized as follows: 1.4 for individuals with low PAL, 1.6 for individuals with moderate PAL, and 1.8 for individuals with high PAL. The three-day food record was utilized to record subjects' daily food intake, and the total calories from food intake were calculated using NutriSurvey version 2007 (EBISpro, Willstaett, Germany). The data were further calculated for basal metabolic rate (BMR) and recommended calorie intake (RCI) (Mardani et al. 2020). The formula is presented in Table 1.

Table 1. Basal metabolic rate and recommended calorie intake formula.

Measurements	Formula
Male BMR	(13.75 x weight (kg)) + 66.5 + (5.003 x height (cm)) – (6.755 x age (years))
Female BMR	(9.563 x weight (kg)) + 655.1 + (1.850 x height (cm)) – (4.676 x age (years))
RCI	BMR x PAL factor

The subjects' total food intake was compared with their recommended calorie intake. The results were then categorized as underconsumption, normal consumption, and overconsumption. Total food intake within 80–100% of the subjects' recommended calorie intake was considered normal consumption (Mardani et al. 2020). Additionally, the Spearman test with a significance of $p<0.05$ was used as the statistical test in this study. Analysis was carried out using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA). The Research Ethics Board of the School of Medicine and Health Sciences, Atma Jaya Catholic University

of Indonesia, Jakrata, Indonesia, granted ethical approval for this study. The information collected in this study was used for research purposes only.

RESULTS

The study revealed that among the 238 preclinical students examined, the group with a moderate level of physical activity exhibited the highest prevalence (70.1%), whilst the group with a high degree of physical activity had the lowest prevalence (7.6%). Table 2 displays the distribution of physical activity levels among the students who participated in this study.

Table 2. Distribution of the participants' physical activity levels.

PAL	n	%
Low	53	22.3
Moderate	167	70.1
High	18	7.6
Total	238	100

PAL: Physical activity levels.

Table 3. Distribution of the participants' total food intake.

TFI	n	%
Underconsumption	124	52.1
Normal Consumption	91	38.2
Overconsumption	23	9.7
Total	238	100

TFI: Total food intake.

Table 4. Crosstabs between physical activity levels and total food intake.

PAL	Total food intake			Total
	Under-consumption (%)	Normal consumption (%)	Over-consumption (%)	
Low	21 (39.6%)	19 (35.8%)	13 (24.5%)	53
Moderate	93 (55.7%)	65 (38.9%)	9 (5.4%)	167
High	10 (55.6%)	7 (38.9%)	1 (5.5%)	18

PAL: Physical activity levels.

Table 5. Association between physical activity levels and total food intake.

Variable	Correlation coefficient	p
PAL	-0.171	0.008

PAL: Physical activity levels.

Table 3 shows the distribution of the subjects' total food intake. The underconsumption group was found to be the most prevalent (52.1%), while the overconsumption group was found to be the least prevalent (9.7%). As shown in Table 4, subjects with a low physical activity level had the lowest prevalence of normal food consumption (35.8%) and the highest prevalence of overconsumption (24.5%). Subjects with moderate and high physical activity levels demonstrated comparable results, characterized by a low prevalence of over-consumption (5.4% and 5.5%, respectively).

Table 5 presents the results of the correlation analysis between physical activity levels and total food intake using the Spearman test. There was a statistically significant association between physical activity levels and total food intake, with a p-value of 0.008. The correlation coefficient of -0.171 indicated a weak negative correlation between the variables.

DISCUSSION

A total of 238 preclinical students were involved in this study, ranging in age from 17 to 23 years old. There were 53 respondents (22.3%) with a low physical activity level, 167 respondents (70.2%) with a moderate physical activity level, and 18 respondents (7.6%) with a high physical activity level. These findings are comparable to data from the World Health Organization, which stated that 28% of adults were not active enough in 2016 globally. Similar studies conducted by Wattanapisit et al. (2016) and Nacar et al. (2015) found that more than half of medical students have low physical activity. This is due to the perception that studying is more important than doing physical activity and that physical activity can decrease academic performance. On the other hand, two other investigations revealed that medical students generally engage in more physical activity than the average person (Stanford et al. 2013, Wattanapisit et al. 2015). This discrepancy between studies happened because not all medical schools provide enough places and policies for medical students to be physically active. Overall, physical activity is affected not only by environmental matters but also by several other factors, such as social and cultural factors (Saimon et al. 2015a, 2015b).

The distribution of total food intake showed that underconsumption (52.1%) was more prevalent among the subjects, followed by normal consumption (38.2%) and overconsumption (9.7%). High food intake among the students was the least prevalent, which might occur due to the students' knowledge of the negative impact of obesity. In the studies carried out by Sheikh et al. (2016) and

Bergeron et al. (2017), they found that social and psychological factors from the environment and efficient nutritional education influenced medical students to adopt healthy eating habits.

The data in this study analyzed using the Spearman test showed a statistically significant association between the students' physical activity levels and total food intake (p=0.008). The analysis showed a weak negative correlation, with a correlation coefficient value of -0.171. It indicated that the relationship between the two variables was opposite or not in the same direction. Therefore, the results can suggest that an increase in physical activity levels causes a decrease in total food intake. This is similar to the findings of previous studies, which found that individuals with high physical activity had stronger appetite regulation so that total food intake was more balanced with energy expenditure (Hopkins et al. 2022, Catenacci et al. 2014, Alkahtani et al. 2014).

Bird & Hawley (2017) stated in their study that muscle contraction during exercise stimulates adenosine monophosphate-activated protein kinase (AMPK) activity. Subsequently, Tre-2, Bub-2, cell division cycle 16 (CDC 16) domain family, member 1 (TBC1D1) will be deactivated, which promotes the translocation of glucose transporter type 4 (GLUT4) to cell membranes and thereby increases glucose uptake. Insulin sensitivity itself is known to play a role in compensatory responses to high energy intake. Jeong et al. (2018) found that pro-opiomelanxocortin (POMC) neurons in the arcuate nucleus have receptors that are similar to those in the transient receptor potential cation channel subfamily V member 1 (TRPV1). An increase in body temperature (from 37 °C to 38 °C) due to exercise or high physical activity will activate the TRPV1-like receptors on POMC neurons, resulting in an anorectic effect that reduces food intake. Slower gastric emptying and a higher release of ghrelin also affect appetite control in populations with high physical activity (Schubert et al. 2014, Horner et al. 2015).

Strength and limitations

This is the first cross-sectional study in Indonesia that specifically presents the correlation between physical activity levels and food intake. This study involved a large number of respondents so that the data obtained could represent the population of medical school students. However, the use of the Baecke Physical Activity Questionnaire and the three-day food record method potentially caused recall bias because, in order to fill out the questionnaire, the participants needed to rely on their memory. This cross-sectional study could not determine whether a low level of physical activity causes more food intake than the total energy requirement or whether the amount of excess food intake causes a low level of physical activity. This study did not investigate other aspects that could potentially become confounding factors, such as sleep quality and psychological stress.

CONCLUSION

Preclinical students with a low physical activity level have the highest prevalence of overconsumption. Physical activity levels and total food intake have a statistically significant relationship with a weak negative correlation. A higher level of physical activity is associated with a reduction in the excessive consumption of total food intake.

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Conflict of interest

None.

Ethical consideration

Research ethics certification No. 10/07/KEP-FKIKUAJ/2020 was issued on 13/7/2020 by the Ethical Clearance Committee of the School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia.

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Author contribution

LEH contributed to the conception and design, the drafting of the article, the final approval of the article, the provision of study materials, the funding, the administrative, technical, or logistical support, and the collection and assembly of data. FT contributed to the conception and design, the analysis and interpretation of the data, the drafting of the article, the critical revision of the article for important intellectual content, the final approval of the article, the provision of study materials, the statistical expertise, and the administrative, technical, and logistic support. NAP and V contributed to the critical revision of the article for important intellectual content and the final approval of the article. NTW contributed to the final approval

of the article, the provision of study materials or patients, and the statistical expertise.

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Original Research Report

HEMOGLOBIN A1c (HbA1c) LEVELS FROM THE EXAMINATION OF DIFFERENT BLOOD PROPORTIONS IN K2EDTA TUBES USING AN ENZYMATIC METHOD

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ABSTRACT

Hemoglobin A1c (HbA1c) examination is the gold standard for diagnosing and monitoring diabetes mellitus patients. In the examination, the pre-analytical phase has the most considerable error rate at 61%. One of the contributing factors to errors in the pre-analytical phase is the use of anticoagulants that does not adhere to established guidelines. Additionally, the incompatibility of the sample volume proportions and the anticoagulants in K2EDTA tubes has been observed in numerous cases. The significance of HbA1c testing, particularly in the prevention of diabetes complications, underscores the need for appropriate procedures to be followed throughout the sampling and pre-analytical phases. This study aimed to determine the effect of differences in the proportion of blood sample volume and anticoagulants in K2EDTA tubes on HbA1c levels. This research was conducted from August to September 2022. The research samples were collected from six healthy subjects at the Department of Medical Laboratory Technology, Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia. The blood samples were divided into K2EDTA tubes with varying volumes of 1 mL, 2 mL, 3 mL, and 4 mL. The HbA1c levels were then examined at the Clinical Chemistry Laboratory of Politeknik Kesehatan Kemenkes Surabaya. The data were analyzed using a one-way ANOVA test. The statistical test results ($p>0.05$) indicated that the proportion of samples containing anticoagulants in the K2EDTA tubes did not have any significant effect on HbA1c levels. In conclusion, it is acceptable to utilize K2EDTA tubes with varying blood sample volumes for measuring HbA1c levels.

Keywords: Diabetes mellitus; hemoglobin A1c (HbA1C); pre-analytical phase

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Highlights:
1. Research on the effect of blood volume proportion in the examination of HbA1c levels using K2EDTA anticoagulant has yet to be widely carried out in Indonesia.
2. The analysis conducted utilizing K2EDTA tubes revealed that the varying proportions of blood samples had no effect on HbA1c levels.
3. This article highlights the necessity of evaluating the pre-analytical phase (sample preparation) of a laboratory process to improve the accuracy of the results and minimize false high or low results in the HbA1c test.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by blood sugar levels exceeding normal limits. This disorder can be caused by damage to pancreatic beta cells or a decrease in the functionality of the pancreatic glands. It may lead to either a complete absence or a diminished production of insulin (Infodatin 2020). Globally, this disease is a leading cause of blindness, heart disease, and kidney failure. The International Diabetes Federation (IDF) estimated that at least 463 million people aged 20–79 years suffered from diabetes in 2019 (International Diabetes Federation 2017). According to the results of an Indonesian basic health research project conducted in 2018, the prevalence of diabetes mellitus among individuals aged 15 years was 2%, as determined by medical diagnosis. The results of blood sugar examinations revealed that the prevalence of diabetes mellitus increased from 6.9% in 2013 to 8.5% in 2018. The presented data indicates that only 25% of individuals diagnosed with diabetes are aware of their condition (Infodatin 2020).

Hemoglobin A1c (HbA1c) is the gold standard for monitoring diabetes mellitus patients. The formation of HbA1c bonds occurs slowly and will decompose along with the age of erythrocytes, which is about 3–4 months (Wulandari et al. 2020). The amount of glycated hemoglobin depends on the amount of available blood glucose. If blood glucose levels increase for a long time, erythrocytes will be saturated with glucose to produce HbA1c (Utomo et al. 2015). Measurement of HbA1c should be carried out routinely for all diabetic patients, both through the initial examination and as part of ongoing care, to prevent further complications in individuals with diabetes. Three factors influence the interpretation of HbA1c examinations: pre-analytical (patient identification, patient preparation, sampling, sample collection, and sample quality), analytical (sample analysis), and post-analytical (laboratory results) (Gupta et al. 2014, Yaqin 2015).

In a study by Yaqin (2015), it was found that HbA1c examinations in Indonesia had the most considerable error rate of 61% during the pre-analytical phase. The study further showed that the error rates during the analytical phase and the post-analytical phase were 25% and 14%, respectively. One of the pre-analytical factors that affects the results of the examination is the administration of anticoagulants. According to the guidelines from the Clinical and Laboratory Standards Institute (CLSI) in 2004, procedures for processing blood specimens in vacutainer tubes containing blood volumes higher or lower than the recommended levels must be rejected. Too little or too much anticoagulant can affect the results of the study. In addition, the difference in blood volume for all types of vacutainers should not be more than 10% of the recommended volume (Dayalan et al. 2020).

According to a study by Cahyani (2018), the proportion of blood samples filled within the K2EDTA vacutainer tube had an apparent effect on the results of complete blood tests. Incompatible proportions of the sample volume with the specified volume standard established by the CLSI are the most common cases regarding HbA1c examinations. The blood volume used in the K2EDTA tube is often less than the standard volume due to various reasons, such as insufficient blood coming out during the blood sampling process. HbA1c examinations play an important role, especially in preventing diabetes complications. Therefore, it is necessary to adhere to the appropriate procedure, which includes the collection and preparation of samples throughout the pre-analytical phase. It can be crucial to obtain accurate examination results in order to improve the quality of service provided to the public. This study aimed to analyze the effect of blood volume proportions in K2EDTA tubes on HbA1c levels as measured using the enzymatic method. Medical laboratory professionals are expected to give more consideration to the pre-analytical phase in order to obtain accurate examination results and provide better health services to the general population.

MATERIALS AND METHODS

This was a descriptive-analytical study with a cross-sectional design. This study was conducted in November 2022 at Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia. The inclusion criteria included healthy people aged 20–30 years. The exclusion criteria included people who were pregnant and had a history of diseases such as anemia, diabetes mellitus, hypertension, kidney failure, and coronary heart disease (Yedla et al. 2015). The samples used in this study were whole blood with four different sample volume treatments but the same amount of anticoagulant in the K2EDTA tubes. A total of six healthy subjects provided the samples for this study. Prior to sampling, the participants filled out an informed consent form. Blood samples were collected from the participants in 1 mL, 2 mL, 3 mL, and 4 mL volumes in EDTA tubes. The samples were homogenized before measuring the level of HbA1c with the enzymatic method using the Photometer 5010 (Robert Riele GmbH & Co., Germany). This study received approval from the Health Research Ethics Committee of the Ministry of Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia, with certificate No. EA/1329/KEPK-Poltekkes_Sby/V/2022 on 14/11/ 2022. The participants in this study were asked to fill out an informed consent form. After the participants gave their consent, blood samples were collected to be used as research samples. The confidentiality of the participants' identities was ensured by assigning a unique code to each research sample. In the event of complications occurring during the sampling process, the researchers bore full responsibility for covering the associated medical expenses.

The method used in collecting samples in this study was consecutive sampling. Federer's formula was employed to estimate the number of sample replications with t=4. The minimum number of sample replications was obtained six times (Sujarweni 2013). The results of the examination were presented in tables. IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, New York, United States) was used to analyze the data. The obtained data were tested for normality using the Shapiro-Wilk test and for homogeneity using the Lavene test. If the data were normally distributed, an ANOVA one-way block design would be employed. If there was at least one pair of different treatments, then the post hoc test was performed to determine

which pair was significantly different. In contrast, if the data were not normally distributed, Kruskal-Wallis test was used (Sujarweni 2013).

RESULTS

Table 1 presents the results of the examination of HbA1c levels according to the quantity of sample proportions and anticoagulants used in the K2EDTA tubes. The average HbA1c level in the 1 mL blood volume sample group collected in the K2EDTA tube was 6.2±0.43. Similarly, the average HbA1c levels were 6.1±0.43 in the 2 mL blood volume sample group, 6.1±0.42 in the 3 mL blood volume sample group, and 6.1±0.52 in the 4 mL blood volume sample group.

Table 1. The results of the examination of HbA1c levels.

Sample code	HbA1c levels (mg/dL)				History of chronic disease
	1 mL of sample	2 mL of sample	3 mL of sample	4 mL of sample	
HOO1	6.7	6.2	6.3	6.5	None
HOO2	5.8	5.8	5.6	5.7	None
HOO3	6.3	6.1	5.9	5.8	None
HOO4	6.7	6.9	6.8	7.0	None
HOO5	6.1	5.7	6.2	5.7	None
HOO6	5.7	5.9	5.8	6.1	None
Mean±SD	6.2±0.43	6.1±0.43	6.1±0.42	6.1±0.52	

This study involved the collection of blood samples from the participants, following predetermined criteria for inclusion and exclusion. Each sample underwent four treatments, consisting of blood volumes of 1 mL, 2 mL, 3 mL, and 4 mL in K2EDTA tubes. The normality test results, as determined by the Kolmogorov-Smirnov test, produced a value that exceeded the significance level of 0.05 (p=0.20). The results suggested that the samples exhibited a normal distribution, therefore prompting the application of a one-way ANOVA statistical test to investigate the potential effect of blood volume proportion on HbA1c levels in a blood examination involving the use of anticoagulant in K2EDTA tubes. The statistical analysis utilizing a one-way ANOVA test revealed a significance value above 0.05 (p=0.20). The test result suggested that there was no significant association between the blood volume proportions and the HbA1c levels from the examination results employing anti-coagulant in the K2EDTA tubes.

DISCUSSION

This study aimed to determine whether varying blood volume proportions in a blood examination using anticoagulants in different K2EDTA tubes affect the HbA1c levels in the collected samples. An HbA1c examination can determine the average blood glucose level in the previous 1–3 months, assess diabetes control to prevent complications, and assess the effectiveness of therapy modifications after 2–3 months. HbA1c that is produced will be stored and remain present in red blood cells for around three months, aligning with the typical life span of these cells (Putri & Larasati 2013, Que et al. 2015). The formation of HbA1c depends on the glucose concentration in the bloodstream, so the results of the HbA1c examination can provide the estimation of the average blood glucose level for around three months. This occurs when hemoglobin, a protein in red blood cells that carries oxygen throughout the body, combines with glucose in the blood to become glycated (Sulistio & Mutiara 2015, Ramadhan & Marissa 2015).

There are several factors that may affect the examination of HbA1c levels, such as the presence of icteric specimens. Icteric specimens are characterized by elevated bilirubin levels (>5.0 mg/dL) and a yellowish color in the serum. This is due to the accumulation of bilirubin in the body, which is indicative of impaired liver function. Hemolysis, the rapid destruction of erythrocytes, can lead to both intrinsic and extrinsic abnormalities in red or reddish erythrocytes and serum. Additionally, conditions characterized by a decrease in red blood cells, such as anemia, thalassemia, and long-term blood loss, can result in falsely decreased levels of HbA1c. Anemia is characterized by a decrease in the hemoglobin level in the blood. A reduction in hemoglobin levels is typically accompanied by a decrease in erythrocyte count and hematocrit levels (Sherwani et al. 2016).

The results of this study differed from those of a study conducted by Sartika & Hestiani (2019), who found a correlation between blood volume proportion and the results of routine blood tests using anticoagulants in K2EDTA tubes. The observed phenomenon can be attributed to differences in osmolarity between the blood and anticoagulant substances. When blood volume is not collected in accordance with the established guidelines for the EDTA vacuum tube, it can result in cellular shrinkage (Sarihati et al. 2019). The findings of this study indicated that the proportion of sample volume did not have any significant effect on the HbA1c examination results when anticoagulants in K2EDTA tubes were used. The absence of effect could be because the HbA1c examination measures glycated hemoglobin, glycohemoglobin, or glycosylated hemoglobin (GHb). HbA1c is a compound formed through the reaction between glucose and hemoglobin, which is the component of red blood cells responsible for the transportation of blood and oxygen throughout the body.

Consequently, the morphology of red blood cells does not influence the results of the examination (Rawal et al. 2016). According to the findings of this study, it is possible to utilize a sample volume of less than 3 mL in a K2EDTA tube, which typically has a standard size of 3 mL, for the purpose of conducting a HbA1C examination.

Strength and limitations

Extensive research regarding the effect of blood volume proportion on HbA1c levels using K2EDTA anticoagulants has not been widely conducted in Indonesia. The findings of this study can serve as a theoretical foundation for understanding the effect of varying proportions of blood samples on the measurement of HbA1c levels. This study also sheds light on the pre-analytical phase, specifically the sample preparation, and its potential to enhance the accuracy of examination results by reducing the occurrence of falsely elevated or low HbA1c levels. The limitation of this study was the inclusion of healthy participants, which may have influenced the observed HbA1c levels in normal ranges. A larger sample size would have been advantageous to mitigate potential biases in the study findings.

CONCLUSION

Varying proportions of blood samples have no significant effect on HbA1c levels in an examination using an anti-coagulant in K2EDTA tubes and an enzymatic method. Therefore, the use of a K2EDTA tube in the examination of HbA1c levels with varying blood sample volumes remains acceptable.

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Conflict of interest

None.

Ethical consideration

The ethical approval for this study was obtained from the Health Research Ethics Committee of the Ministry of Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia, with certificate No. EA/1329/KEPK-Poltekkes_Sby/V/2022 on 14/11/2022.

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Author contribution

M conceptualized and designed the study, analyzed and interpreted the data, drafted the research article, and critically revised the article for important intellectual content. MHN provided the final approval of the article, study materials, and statistical expertise, as well as secured funding for the research. RW offered comprehensive support in administrative, technical, and logistical aspects while also gathering and organizing the data.

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Original Research Report

METFORMIN EFFECTIVENESS IN REDUCING MORTALITY OF COVID-19 PATIENTS WITH TYPE 2 DIABETES MELLITUS AT A TERTIARY HOSPITAL IN INDONESIA

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ABSTRACT

COVID-19 patients with comorbidities, such as type 2 diabetes (T2DM), have a higher mortality rate compared to those without any comorbidities. T2DM patients usually receive metformin as their first-line treatment. However, the effectiveness of metformin in reducing mortality rates still requires further analysis. The objective of this study was to analyze the effectiveness of metformin in reducing mortality rates among COVID-19 patients with T2DM. An analytical observational design with a retrospective cohort approach was used in this study. Samples were acquired from hospitalized COVID-19 patients with T2DM medical records at Fatmawati Central General Hospital, Jakarta, Indonesia, from 2020 to 2021. The samples were collected using a purposive sampling technique and analyzed using Chi-square test ($p<0.05$; $RR<1$). This study comprised 137 samples, with 56 of which received metformin and 81 of which did not receive metformin. The mortality rate in the sample group that received metformin was lower (19.6%) compared to the group that was not given the medication (38.3%). The Chi-square test results indicated a statistically significant relationship between metformin treatment and a lower mortality rate among COVID-19-contracted individuals with T2DM ($p=0.020$; $RR=0.513$). Therefore, this study concludes that the administration of metformin reduces mortality among COVID-19 patients with T2DM.

Keywords: Comorbidity; COVID-19; metformin; mortality; type 2 diabetes mellitus (T2DM)

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Highlights:
1. As there is a scarcity of publications on the use of metformin for COVID-19 in Indonesia, the findings of this present study may contribute more insight to the existing body of research and provide data specific to the Southeast Asian population.
2. This study revealed a decreased mortality rate associated with metformin use in diabetic patients with mild to moderate COVID-19 infection.
3. This study suggests that diabetic patients may continue metformin treatment during a COVID-19 infection as the medication has sustained therapeutic effects.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) was initially discovered in Wuhan, China. It is an infectious disease brought on by the SARS-CoV-2 virus (Centers for Disease Control and Prevention 2023). Indonesia has witnessed a substantial number of reported positive cases and a high mortality rate associated with this particular disease. According to a national report in May 2023, the total number of positive cases recorded nationwide was 6,807,513, while the region of Jakarta accounted for 1,408,154 cases. In South Jakarta, a total of 322,317 cases were officially confirmed up until May 2023, with 3,247 fatalities reported (Satgas COVID-19 2022). The presence of comorbidities, such as type 2 diabetes mellitus (T2DM), is one of the factors contributing to the increased mortality rate in COVID-19 patients. According to the preliminary data obtained from Fatmawati Central General Hospital, Jakarta, Indonesia, it was found that 16 patients who were diagnosed with COVID-19 also had T2DM. Furthermore, the data reported that 68.8% of these patients were deceased (Kshanti et al. 2020, Harbuwono et al. 2022). COVID-19 patients with T2DM reportedly accounted for 36.4% of the positive cases in Jakarta, according to the data on 738 cases. The mortality rate among these patients was reported to be 11.9% (Satgas COVID-19 2022).

Metformin is typically prescribed as a first-line treatment for individuals diagnosed with T2DM due to its ability to lower blood glucose levels. This medication is a therapeutic agent for hyperglycemia by enhancing insulin sensitivity in the body. The mechanism to increase body sensitivity toward insulin is possible through the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) and the translocation of glucose transporter type 4 (GLUT4), thus facilitating an increased uptake of glucose into the cells. In addition, metformin has an anti-inflammatory effect that works by suppressing the release of cytokines and chemokines, such as interleukin 6 (IL-6), interleukin 1 beta (IL-1β), and tumor necrosis factor beta (TNF-β). This suggests that metformin may have a potential therapeutic advantage that suppresses COVID-19 inflammation in T2DM patients who have contracted the infectious disease (Kamyshnyi et al. 2021).

Previous studies have investigated the effectiveness of metformin in COVID-19 patients with T2DM. A study found a lower mortality rate in patients who received metformin (3%) than those who did not (11%). The study included 328 COVID-19 patients with T2DM, with a categorization of severe (31.4%) and non-severe COVID-19 (68.6%) (Jiang et al. 2021). A separate study with 6,256 samples also yielded comparable findings in female patients (HR=0.785; CI=0.650–0.951; p=0.015). However, no significant association was observed in male patients (HR 0.967; CI 0.82-1.14; p = 0.871) (Bramante et al. 2021). In another study with a sample size of 1,213 and a COVID-19 categorization of mild and severe, it was found that the administration of high-dose metformin increased acidosis incidence. Nevertheless, the study did not find any significant effect on patient mortality (adjusted HR=0.87; 95% CI=0.36–2.12; p=0.757) (Cheng et al. 2020). The aforementioned studies have provided evidence about the effectiveness and effects of metformin in COVID-19 patients with T2DM.

The side effects of metformin pose a challenge for healthcare professionals to evaluate its potential benefits and risks. This underlines the need for further research into the effectiveness of metformin, particularly in reducing mortality rates among COVID-19 patients with T2DM. Such studies, however, remain scarce in Indonesia. The objective of this study was to assess the effectiveness of metformin in lowering mortality among COVID-19 patients with T2DM at Fatmawati Central General Hospital, Jakarta, Indonesia. The findings of this study may be helpful in providing valuable insights into the improved management of COVID-19 and T2DM.

MATERIALS AND METHODS

This study employed an analytical observational design with a retrospective cohort method. Data were collected from the medical records of 137 COVID-19 patients with T2DM who were hospitalized at Fatmawati Central General Hospital between 2020 and 2021. Fatmawati Central General Hospital is located in South Jakarta, Indonesia. The sample size was determined using a two-population proportion hypothesis test, resulting in a minimum requirement of 108 samples. This study used a purposive sampling technique. The inclusion criteria were T2DM patients who were diagnosed with mild to moderate COVID-19 and within the age range of 20–85 years. The patients included in this study were either receiving metformin as monotherapy, as part of a combination therapy, or were not receiving metformin treatment at all. The clinical classification of COVID-19 severity was determined according to the COVID-19 Management Guidelines (Burhan et al. 2022). Patients with comorbidities other than T2DM (e.g., cardio-vascular disease, cerebrovascular disease, chronic obstructive pulmonary disease, and chronic kidney disease) and immunocompromised patients were excluded from this study.

The independent variable was the administration of metformin, while the dependent variable was patient

mortality. The data collected in this study comprised several patient characteristics, such as gender, age, clinical classification of COVID-19 severity, body mass index (BMI), as well as the administration of metformin and other medications. The data were then documented and organized with Microsoft Excel for Windows, version 16.0 (Microsoft Inc., Redmont, WA, USA). The statistical analyses were performed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, N.Y., USA). The frequency data were presented using tables. The Chi-square test was used in the analysis of the relationship between the dependent and independent variables. A significance level of $p<0.05$ indicated a significant relationship (Sutriyawan 2021). This study obtained permission from the Research Ethics Committee of the Faculty of Medicine, Universitas Pembangunan Nasional "Veteran" Jakarta, Jakarta, Indonesia, with approval letter No. 76/IV/2023/ KEPK on 11/4/2023.

RESULTS

After applying the predetermined inclusion and exclusion criteria, a total of 202 patient data sets were collected from medical records. Additional screening was conducted, resulting in the exclusion of 65 data sets due to incomplete information. A total of 137 data sets were deemed eligible for analysis.

Table 1 displays the characteristics of T2DM patients who were diagnosed with COVID-19. The majority of the patients exhibited characteristics such as advanced age, male gender, moderate symptoms, and obesity. There were more patients who did not receive metformin treatment compared to those who did, showing that the non-metformin group accounted for the majority of total samples. The patients' characteristics showed no statistically significant difference between the metformin and non-metformin groups ($p>0.05$).

Among the 137 COVID-19 patients with T2DM in this study, only 56 individuals received metformin treatment. Table 2 shows the distribution of metformin administration among these patients. Only six patients received metformin as monotherapy, while the remaining patients received the medication as part of combination therapy.

Table 3 categorizes the administration of medications other than metformin. The majority of the patients were prescribed antidiabetic drugs, with insulin being the most commonly used medication. The results showed that there were no statistically significant differences between groups in terms of the administration of medications other than metformin ($p>0.05$).

Table 1. Characteristics of patients in the metformin and non-metformin groups.

Characteristics	Frequency [n (%)]		%	p
	Metformin group [n=56 (40.9%)]	Non-metformin group [n=81 (59.1%)]		
Age				>0.05
20–39 y.o.	6 (10.7)	10 (12.3)	11.7	
40–49 y.o.	8 (14.3)	15 (18.5)	16.8	
50–59 y.o.	15 (26.8)	30 (37.0)	32.8	
60–69 y.o.	22 (39.3)	20 (24.7)	30.7	
70–79 y.o.	4 (7.1)	5 (6.2)	6.6	
≥80 y.o.	1 (1.8)	1 (1.2)	1.5	
Sex				>0.05
Male	30 (53.6)	40 (49.4)	51.1	
Female	26 (46.4)	41 (50.6)	48.9	
COVID-19 severity				>0.05
Mild	21 (37.5)	37 (45.7)	42.3	
Moderate	35 (62.5)	44 (54.3)	57.7	
BMI				>0.05
Underweight	3 (5.4)	1 (1.2)	2.9	
Normal	17 (30.4)	26 (32.1)	31.4	
Overweight	10 (17.9)	20 (24.7)	21.9	
Obesity	26 (46.4)	34 (42.0)	43.8	

Table 2. Distribution of metformin administration as monotherapy and as part of combination therapy.

Category	Frequency [n (%)] [n=56 (40.9%)]	%
Monotherapy	6 (10.7)	4.4
Part of combination therapy	50 (89.3)	36.5

Table 3. Distribution of the administration of medications other than metformin.

Characteristics	Frequency [n (%)]		%	p
	Metformin group [n=56 (40.9%)]	Non-metformin group [n=81 (59.1%)]		
Antidiabetics				>0.05
Thiazolidinedione	0 (0.0)	1 (100.0)	0.7	
DPP-4 inhibitor	0 (0.0)	1 (100.0)	0.7	
Sulfonylurea	7 (30.4)	16 (69.6)	16.8	
α-glucosidase inhibitor	1 (33.3%)	2 (66.7)	2.2	
Insulin	48 (42.1)	66 (57.9)	83.2	
Antiviral	52 (43.0)	69 (57.0)	88.3	> 0.05
Antibiotic	53 (41.1)	76 (58.9)	94.2	> 0.05
Anticoagulant	42 (40.8)	61 (59.2)	75.2	> 0.05
Antifungal	4 (25.0)	12 (75.0)	11.7	> 0.05
Glucocorticoid	36 (45.6)	43 (54.4)	57.7	> 0.05

Notes: DPP-4=Dipeptidyl peptidase-4.

Table 4 presents the distribution of mortality and survival among the total patients in this study. Among the 137 COVID-19 patients with T2DM, a total of 42 patients (30.7%) succumbed to the disease. The majority of the patients, comprising 95 individuals (69.3%), survived following the COVID-19 infection.

Table 4. Distribution of patient mortality and survival.

Category	Frequency (n)	%
Mortality	42	30.7
Survival	95	69.3

Table 5 displays the results of the Chi-square test. The analysis was performed to assess the correlation between metformin administration and patient mortality. The results demonstrated a statistically significant correlation between the administration of metformin and a lower mortality rate among COVID-19 patients with T2DM ($p<0.005$; $RR<1.000$).

Table 5. Results of the Chi-square test of metformin administration and patient mortality.

Groups	Mortality [n (%)]	Survival [n (%)]	p	RR (95% CI)
Metformin	11 (19.6)	45 (80.4)	0.020	0.513 (0.282–0.933)
Non-metformin	21 (38.3)	50 (61.7)		

Notes: RR=Risk ratio.

DISCUSSION

The age grouping used in this study adhered to the same categorization as outlined in the study conducted by Woolcott & Castilla-Bancayán (2021). Patients under the age of 20 were excluded from this study because of the low prevalence of T2DM within this particular age group. The results of this study showed that the majority of the COVID-19 patients with T2DM were concentrated in the age group of 50–59 years (32.8%). According to a study conducted by El Chakhtoura et al. (2017), aging influences the decrease in immune cell recruitment functionality. This particular factor may lead to a higher susceptibility to developing diseases among older adults.

This study showed that there was a higher proportion of male patients in comparison to female patients. This is consistent with the study conducted by Luo et al. (2020), in which the majority of COVID-19 patients with T2DM were male (55.1%). This occurrence could potentially be related to the different behavioral patterns exhibited by men and women. A previous study revealed that women demonstrated a higher prevalence of hygiene habits and adopted healthier lifestyles compared to men. It is possible that this could make men more susceptible to infections (Bwire 2020).

The clinical categorization of the COVID-19 patients in this study showed that the majority of them exhibited moderate symptoms. Yitao et al. (2021) conducted a study that revealed a comparable finding, wherein the prevalence of moderate-severity cases was 12.2% among COVID-19 patients with T2DM. This study reported a higher number of patients with a moderate clinical classification of COVID-19 since Fatmawati Central General Hospital served as a referral center for COVID-19 cases during the pandemic.

The present investigation revealed a high prevalence of obesity among the patient population. Individuals who have diabetes mellitus and obesity have a 4.5 times greater risk of hospitalization compared to those without both conditions Giorgino et al. (2021). According to Holly et al. (2020), COVID-19 patients who have T2DM and obesity commonly experience impairments in immune system functioning. These impairments include disruptions in the activity of T lymphocytes and natural killer cells, which subsequently lead to the development of more severe clinical manifestations.

Most of the patients in this study did not receive metformin treatment. This is consistent with the study by Jiang et al. (2021), who showed that most COVID-19 patients with T2DM did not receive metformin (69.5%). In addition to its immunomodulatory effects, metformin has anti-inflammatory effects through the suppression of cytokines such as IL-6, IL-1β, and TNF-β. Metformin is the first line of T2DM treatment because it can reduce blood glucose levels by phosphorylating AMPK and translocating GLUT4, which can enhance the uptake of glucose into the cells. This medication is administered to T2DM patients who suffer from infections. However, COVID-19 patients generally experience gastrointestinal disorders such as nausea, vomiting, and diarrhea. These manifestations are similar to the adverse effects associated with metformin administration. Therefore, it is imperative to carefully evaluate the patient's state before administering metformin to prevent the onset of worsening symptoms (Groff et al. 2021). This observation may clarify the disparity in the number of patients between the metformin and non-metformin groups.

Insulin was the most widely used antidiabetic treatment in this study. Luo et al. (2020) observed a similar finding among COVID-19 patients with T2DM. In the study, 53.7% of the patients received insulin therapy. Insulin doses can be modified quickly to provide rapid relief from hyperglycemic conditions. Therefore, insulin is frequently used in hospitals for the management of diabetes mellitus (Inzucchi 2023). Other medications used in this study were antivirals, antibiotics, anticoagulants, antifungals, and glucocorticoids. SARS-CoV-2 is

the viral pathogen responsible for the COVID-19 infection. Thus, antiviral therapy is the most optimal approach for its treatment. In a recent trial, antiviral medications were administered to 89.9% of the total samples (Wang et al. 2020). It is worth noting that COVID-19 patients may also receive antibiotic treatment. Viral infections are commonly accompanied by bacterial co-infections (Kristanti et al. 2022). According to the research conducted by Wang et al. (2021), a significant proportion of COVID-19 patients, specifically 98%, received antibiotic treatment. In addition, COVID-19 patients with T2DM frequently experience hyper-coagulation, which consequently requires the administration of anticoagulant medications. According to prior research, 30.7% of diabetic patients diagnosed with COVID-19 received anticoagulants (Luo et al. 2020, Carfora et al. 2021). Diabetes is a risk factor for the development of fungal infections. Meanwhile, COVID-19 increases the susceptibility to fungal infections through endothelial dysfunction (John et al. 2021). In the study by Zhang et al. (2020), it was shown that 12.2% of patients required antifungal medication. The study additionally revealed that 41% of patients received glucocorticoid therapy. Glucocorticoids play a substantial role in COVID-19 treatment due to their anti-inflammatory effects. However, glucocorticoid administration may cause hyperglycemia, so periodic monitoring is necessary (Alexaki & Henneicke 2021).

This study revealed that the majority of the patients survived. Similar results were observed in a recent study, with a survival rate of 57.7% and a mortality rate of 42.3% among the patients (Mirani et al. 2020). Conversely, the study by Harbuwono et al. (2022) discovered that COVID-19 patients with T2DM had a higher mortality rate (21.3%) compared to those without comorbidities (2.8%). Diabetic patients generally experience chronic inflammation due to their hyperglycemic condition. They may exhibit an increased inflammatory response when infected with SARS-CoV-2 (Kamyshnyi et al. 2021). COVID-19 patients with T2DM can notice improved clinical symptoms when their blood glucose levels are properly controlled. However, the researchers did not record the blood glucose levels of the subjects during the course of this study. Future research is required to validate the hypothesis regarding the relationship between blood glucose regulation and patient mortality.

The Chi-square test in this study revealed a significant relationship between a reduced number of fatalities and metformin treatment among COVID-19 patients with T2DM. In a previous study, a relationship was found between metformin therapy and a mortality reduction among the research subjects (p=0.01) (Luo et al. 2020). Several other studies have reported similar findings regarding metformin administration and patient mortality. An association between metformin treatment and mortality rate was demonstrated among the patients of a study (OR=0.64, 95% CI=0.51–0.79) (Yang et al. 2021). Furthermore, the study by Lally et al. (2021) concluded that metformin administration to COVID-19 patients with T2DM resulted in a reduced mortality rate over a 30-day period. Metformin can provide anti-inflammatory effects by suppressing nuclear factor kappa B (NF-κB) signaling. NF-κB functions to express cytokines that can activate the inflammatory response. Through the suppression of NF-κB activity, the patient's body will have a milder inflammation response, resulting in the alleviation of symptoms. Metformin also acts as an immunomodulator by promoting the production of M2 macrophages, regulatory T cells, and cytotoxic T lymphocytes (CD8+ memory T cells). The immunomodulatory action of metformin in COVID-19 patients with T2DM may aid the immune response against the SARS-CoV-2 virus. Metformin has been observed to enhance the activation and phosphorylation of AMPK, which can stabilize angiotensin-converting enzyme 2 (ACE2) receptors by preventing the degradation of proteasomes. A stable structure of the ACE2 receptor may potentially lower the probability of the SARS-CoV-2 spike protein binding to it (Kamyshnyi et al. 2021).

The study demonstrated a significant relationship between metformin administration and reduced mortality rates among COVID-19 patients with T2DM. However, it was also necessary to conduct a comparison between metformin and other medications used for the treatment of diabetes. Prior research has found an association between the administration of insulin and higher mortality rates among COVID-19 patients with T2DM. Sulfonylurea monotherapy was shown to have inconclusive effects, while a combination therapy with metformin showed promising results in lowering mortality (Kan et al. 2021, Ouchi et al. 2022). The effectiveness of monotherapy with α-glucosidase inhibitors yielded ambiguous results. However, a combination therapy with metformin demonstrated more favorable outcomes (Li et al. 2022, Nguyen et al. 2022). The findings of a prior study revealed a lack of correlation between thiazolidinedione administration and patient mortality. Meanwhile, the administration of DPP4-inhibitors produced inconclusive results either in monotherapy or combination therapy, especially with metformin (Chen et al. 2022). Overall, metformin surpassed other diabetes treatments in lowering the mortality rate of COVID-19 patients with T2DM.

Strength and limitations

This study provides information regarding the use of

metformin treatment in reducing the mortality rate among COVID-19 patients with T2DM. However, further research in Indonesia is necessary given the scarcity of published studies on this subject matter. Further studies need to evaluate blood glucose and HbA1c levels in correlation with patient mortality, variables that were not assessed in this study. Furthermore, insulin resistance is important to explore in future studies, considering that the use of insulin is high among COVID-19 patients with T2DM.

CONCLUSION

Metformin, as a first-line treatment for hyperglycemia, is related to decreased mortality rates of COVID-19 patients with T2DM. The administration of metformin is beneficial for COVID-19 patients with T2DM who are at a higher risk of mortality because of their comorbidity. A combination therapy using metformin and other medications continues to result in a notable decrease in mortality rates among COVID-19 patients with T2DM.

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Conflict of interest

None.

Ethical consideration

The study was ethically approved on 11/4/2023 by the Research Ethics Committee of the Faculty of Medicine, Universitas Pembangunan Nasional "Veteran" Jakarta, Jakarta, Indonesia, with approval letter No. 76/IV/2023/KEPK.

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Author contribution

YZ contributed to the conception and design, analysis and interpretation of the data, drafting of the article, and statistical expertise. HY, UH, EH, and DB contributed to the critical revision of the article for important intellectual content, final approval of the article, and statistical expertise. MIM contributed to the critical revision of the article for important intellectual content, final approval of the article, provision of study materials, and statistical expertise.

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Original Research Report

ANTIFUNGAL ACTIVITY OF ENDOPHYTIC BACTERIA ISOLATED FROM MIANA PLANTS (*Coleus scutellarioides* (L.) Benth.) AGAINST *Candida albicans*

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ABSTRACT

Candida albicans is the most common organism responsible for both mucosal and systemic infections, accounting for approximately 70% of fungal infections worldwide. Miana, scientifically known as *Coleus scuttellarioides* (L.) Benth., is recognized for its use in traditional medicinal practices. Miana plants contain endophytic bacteria that possess the ability to produce secondary metabolites with potential antifungal agents. The objective of this study was to assess the antifungal activity of nine endophytic bacteria isolates derived from *Coleus scuttellarioides* against *Candida albicans*. This study was laboratory-based qualitative experimental research that applied the Kirby-Bauer diffusion method and several modifications. The *Candida albicans* specimens were spread throughout the entire potato dextrose agar medium. Afterward, paper discs that had been soaked in a liquid culture of endophytic bacterial isolates were carefully placed on the surface of the medium. The complete setup was then incubated for 1–2 days. The potential antifungal activity of endophytic bacteria was assessed by observing the emergence of a clear zone surrounding their growth, which would indicate inhibition. An additional observation was performed in the follow-up test, involving the use of Sabouraud dextrose agar medium to confirm the initial test result. The results from the inhibitory test revealed that none of the bacterial isolates exhibited any inhibition zone. Conversely, ketoconazole as the positive control showed an inhibition zone with an average diameter of 28.5 mm. In conclusion, endophytic bacterial isolates obtained from *Coleus scuttellarioides* have no discernible antifungal properties against *Candida albicans*. This study implies that ketoconazole remains effective in treating infections caused by *Candida albicans*.

Keywords: *Candida albicans*; *Coleus scutellarioides* (L.) Benth.; endophytic bacteria; human and health; tropical disease

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Highlights:
1. Due to the adverse effects associated with current antifungal drugs, research on traditional medicine is necessary to explore other options for candidiasis treatment.
2. Following the findings of this study, it is recommended to conduct further research by incorporating *Coleus scuttellarioides* filtrate into the growth medium, since this may improve bacterial growth by producing optimal secondary metabolites.

INTRODUCTION

Candidiasis, an infection caused by *Candida*, is the most prevalent fungal infection in the world. Of all *Candida* species, *Candida albicans* is the most common cause of mucosal and systemic infections, accounting for around 70% of all fungal infections worldwide (Bhateja 2018, Talapko et al. 2021). Candidiasis is one of the most frequently found fungal infections in tropical countries, such as Indonesia. High temperatures and humidity may contribute to this health issue (Makhfirah et al. 2020). *Candida* is a commensal fungus that typically lives on the skin and within several parts of the human body, including the mouth, throat, intestines, and vagina. This microbe is a normal flora when present in normal amounts. However, *Candida* may cause infection when it experiences uncontrolled growth or when the immune system of the host is compromised (Romo & Kumamoto 2020). In the presence of predisposing factors, *Candida albicans* can undergo a transition to become a pathogen,

resulting in the development of a candidiasis infection.

A study conducted between 2013 and 2016 at the Mycology Division of the Skin and Genital Health Outpatient Unit, Dr. Soetomo General Academic Hospital in Surabaya, Indonesia, revealed a rise in candidiasis cases. The study identified a yearly increase in the number of patients diagnosed with candidiasis, with a total of 99 patients (6.23%) in 2013, 77 patients (6.08%) in 2014, 55 patients (5.85%) in 2015, and 67 patients (8.97%) in 2016 (Puspitasari et al. 2019). In the study conducted by Maulana et al. (2019), it was observed that the prevalence of invasive candidiasis in the Intensive Care Unit at Dr. Hasan Sadikin Central General Hospital, Bandung, Indonesia, during the period of June 2016 to June 2017 was 3.5%. Furthermore, the investigation revealed an exceptionally high mortality rate of 81.8% associated with this condition. According to data provided by the Maluku Provincial Health Office, the number of candidiasis cases among individuals aged 15–24 years in Ambon City, Indonesia, showed fluctuations over the years. Specifically, there were 129 cases reported in 2014, followed by an increase to 196 cases in 2015, and a subsequent decrease to 129 cases in 2016 (Tidore 2018).

One of the drugs commonly used to overcome fungal infections is ketoconazole. However, ketoconazole is known to have side effects such as pruritus, nausea, rash, abdominal pain, headache, dizziness, fatigue, impotence, menstrual disorders, and gynecomastia (Lieberman & Curtis 2018, Sinawe & Casadesus 2023). Considering these side effects, people are increasingly opting for alternative treatments that incorporate natural ingredients or traditional medicinal practices. Miana is among the botanical species used in traditional medicinal practices. Scientifically known as *Coleus scutellarioides* (L.) Benth., miana is a native botanical species originating from India and Thailand. Miana plants reproduce vegetatively and are easy to find in various places. The plants are also endemic in Maluku, Indonesia, and are widely used by the local people to treat many different ailments, such as typhoid, cough, menstrual pain, boils, and fever (Wakhidah & Silalahi 2018).

It is noteworthy that multiple studies have demonstrated the presence of beneficial characteristics in these particular plants. The studies carried out by Muljono et al. (2016) and Setianingrum et al. (2014) observed and documented the antibacterial and antifungal activities of miana plants. Other studies have found evidence regarding the anti-inflammatory effects of the plants. It was reported that miana plants contain antioxidant and anti-diabetic compounds (Marpaung 2014, Levita et al. 2016, Novanti & Susilawati 2017). Similar to other plant species, miana plants typically contains endophytic bacteria, which can produce secondary metabolites that resemble those of their host plants. Consequently, these endophytic bacteria have potential as antifungal agents (Yunita et al. 2022).

Endophytic bacteria refer to a group of microbes that colonize the tissues of healthy plants. However, the bacteria exist in a state of commensalism without eliciting any symptoms or inflicting harm on their hosts (Wu et al. 2021, Yunita et al. 2022). Endophytic bacteria are capable of producing secondary metabolites, including saponins, alkaloids, and terpenoids. In addition, they possess the ability to synthesize cell wall-degrading enzymes, such as chitinase, which exhibit antibiosis properties and are able to function as antifungal agents (Yunita et al. 2016, Vinayarani & Prakash 2018, Foeh et al. 2019). Several researchers have isolated a range of endophytes and conducted experiments to assess their abilities against *Candida albicans*. Rachman & Sari (2020) demonstrated the inhibitory effect of endophytic bacteria derived from *Lannea coromandelica* (Houtt.) Merr. on the growth of *Candida albicans*. Moreover, a recent study documented the presence of endophytic bacteria, classified as isolate K3, within the rhizome of *Curcuma longa*. The bacterial isolates showed high antioxidant properties, indicating their potential as a strong antimicrobial agent against *Candida albicans* (Sulistiyani et al. 2016). Nevertheless, there is currently a lack of research exploring the inhibitory potential of endophytic bacteria derived from miana plants (*Coleus scuttellarioides*) against *Candida albicans*.

In a prior study conducted by Mahulette (2022), a total of nine endophytic bacterial isolates were obtained from the leaves, stems, and roots of miana plants. The isolates were assigned the codes A2, A3, A4, B1, B2, B4, D2, D3, and D4. The examined isolates have demonstrated the capacity to inhibit the growth of *Salmonella typhi*, as evidenced by the largest inhibition zone diameter of 27.5 mm, indicating a robust inhibitory effect. Given the findings of the previous investigation, which indicated the strong antibacterial activity of endophytic bacterial isolates obtained from miana plants, it is critical to identify and assess the antifungal capacity of these isolates as well. Hence, the primary objective of this study was to examine the inhibitory potential of endophytic bacteria obtained from miana plants against *Candida albicans*. This research offers an initial step towards the future development of these isolates as effective antifungal agents.

MATERIALS AND METHODS

The study was conducted from the 29th of December 2022 to the 4th of January 2023 at the Maluku Provincial Health Laboratory and Medical Device Calibration Center, Ambon, Indonesia. This study used a true experimental laboratory research design and an agar diffusion method. The ethical clearance was issued by the Department of Medical Education, Faculty of Medicine, Universitas Pattimura, Ambon, Indonesia, with reference No. 173/FK-KOM.ETIK/VIII/2022 on 22/12/2022. This study was conducted as a continuation of the previous study by Mahulette (2022) on the potential inhibitory effect of bacterial isolates derived from miana plants against *Salmonella typhi*.

The main materials used in this study were nine endophytic bacterial isolates derived from miana plants (*Coleus scuttellarioides* (L.) Benth.). These bacterial isolates were acquired from the previous study conducted by Mahulette (2022). The endophytic bacteria were initially re-cultured by transferring them onto a new nutrient agar medium produced by Merck KGaA (Darmstadt, Germany) with batch number 105540. Afterwards, the cultures were incubated at a temperature range of 28–30 °C for 24 hours (Jamilatun et al. 2020). Meanwhile, the pathogenic fungus used in this study was *Candida albicans* ATCC 90029, sourced from the Maluku Provincial Health Laboratory and Medical Device Calibration Center, Ambon, Indonesia (-3°69' 32.6"S, 128°19'82.4"E). The process of re-culturing *Candida albicans* involved the inoculation of the fungi on a petri dish using Sabouraud dextrose agar medium produced by Merck KGaA (Darmstadt, Germany) with batch number 146028. Subsequently, the petri dish was placed in an incubator set at a temperature of 37 °C for a duration of 24 hours (Zuraidah et al. 2021).

Analyses of *Candida albicans* were performed by including both macroscopic and microscopic observations of the fungus. The growth of the fungal culture in Sabouraud dextrose agar medium was assessed through macroscopic observations, which involved examining the color and surface characteristics of the colonies. *Candida albicans* was characterized by yeast-like colonies and a yellowish-white coloration. Additionally, the surface of the colonies was seen to be wet and convex in nature (Indrayati & Sari 2018). Microscopic observations were conducted after Lugol's solution staining. Then, the preparations were observed under a microscope with 100X magnification (Sulmiyati et al. 2019).

The inhibition test was carried out using the disc diffusion method. The suspension of *Candida albicans* ATCC 90029 was spread over Sabouraud dextrose agar media using a sterilized swab. Blank paper discs were soaked in endophytic bacteria isolate suspension and a control solution (Sulistrioningsih et al. 2020). The experimental setup included the use of a ketoconazole solution with batch number 4591 as the positive control. Additionally, distilled water served as the negative control (Maulana et al. 2020). After a 24-hour incubation period, the measurement of the generated inhibition zone was conducted. The measurement of the inhibition zone was calculated using a defined formula that considered three key points: the vertical diameter (DV), horizontal diameter (DH), and paper disc diameter (DC) (Alioes & Kartika 2019, Winastri et al. 2020).

$$\frac{(DV - DC) + (DH - DC)}{2}$$

RESULTS

The macroscopic observations of *Candida albicans* ATCC 90029 after 48 hours of incubation revealed colonies with a yellowish-white coloration and round, moist, and convex surfaces (Figure 1). The microscopic observations of *Candida albicans* were conducted at 100X magnification. The findings from the observations indicated the presence of cells with an oval shape and a purple color. In addition, it was observed the presence of budding yeast cells, which exhibited asexual reproductive characteristics (Figure 2).

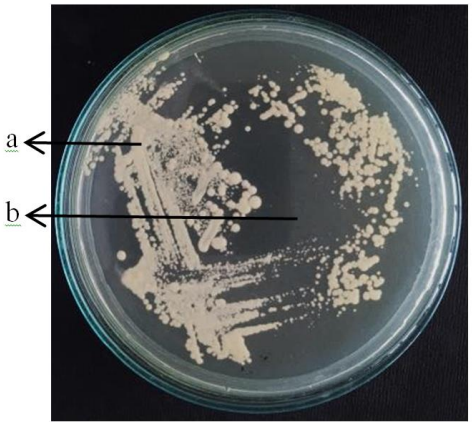

Figure 1. Macroscopic appearance of *Candida albicans* after 48 hours of incubation. a) *Candida albicans* colonies; b) Sabouraud dextrose agar media.

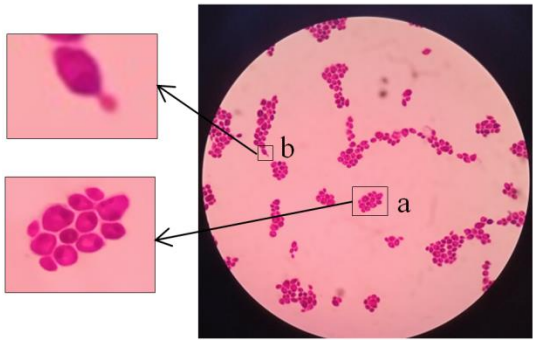

Figure 2. Microscopic observations of *Candida albicans*. a) *Candida albicans* colonies under the microscope; b) A single cell of *Candida albicans*.

The observation of the inhibition zone was conducted after incubating the petri dish for 48 hours. The results obtained from the observations indicated that the bacterial isolates tested did not exhibit any inhibition against *Candida albicans*. This was proven by the absence of a clear zone around the disc paper containing endophytic bacteria in all replicates. The positive control, ketoconazole, had significant inhibitory activity in all replicates, as seen by inhibition zone diameters of 26 mm, 30 mm, and 29.5 mm. In contrast, the negative control, distilled water, failed to show any inhibition zone (Figure 3).

Upon the completion of the initial inhibition test, which yielded no observable inhibitory activity, a follow-up re-inhibition test was conducted with a different method. The bacterial diffusion method was employed with the aim of confirming the earlier findings. Instead of using paper discs, pure cultures of endophytic bacteria were taken using a sterile cotton swab. These cultures were then placed onto Sabouraud dextrose agar media that had been previously inoculated with *Candida albicans*. The data obtained after 48 hours of incubation indicated the same results as the initial inhibition test, where there was no clear zone around the endophytic bacteria.

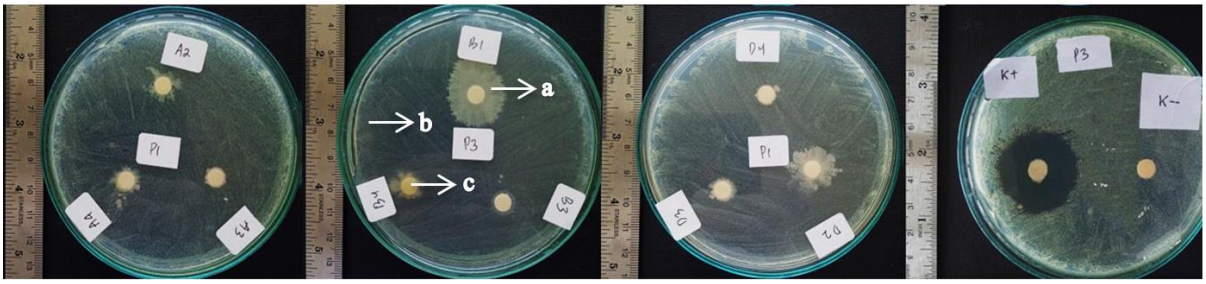

Figure 3. Results of inhibition test of nine endophytic bacterial isolates (isolates A1, A2, A3, B1, B2, B3, D1, D2, and D3) after 48 hours of incubation. a) Representation of endophytic bacteria; b) Population of *Candida albicans*; c) Disc paper used for the experiment. Notes: K(+): Ketoconazole as the positive control; K(-): Distilled water as the negative control.

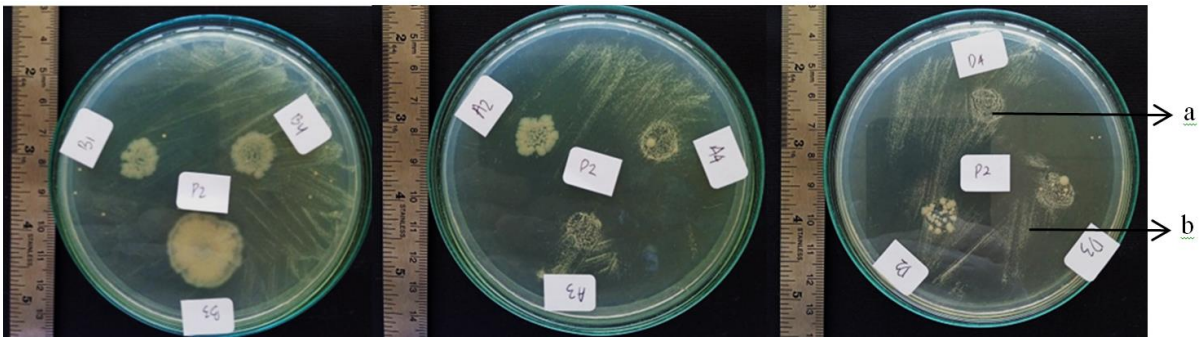

Figure 3. Re-inhibition test after 48 hours of incubation. a) Representation of endophytic bacteria; b) Population of *Candida albicans*.

DISCUSSION

The findings gathered from the tests and observations conducted in this study indicated that the bacterial isolates derived from miana plants were unable to inhibit the growth of *Candida albicans* ATCC 90029. Previous research conducted by Mahulette (2022) proved that endophytic bacteria derived from miana plants possess the capability to inhibit the growth of *Salmonella typhi*. However, it was discovered that the bacteria showed an inability to hinder the proliferation of *Candida albicans*. This might be attributed to the differences in the structure of bacteria and fungi. Fungi comprise both a cell wall and a plasma membrane, making them structurally thicker compared to bacteria, which only contain a cell wall. The cell wall of *Candida albicans* is characterized by its complex structure, with 100–400 nm in thickness. It is composed of five distinct layers stacked in an outer-to-inner arrangement. These layers include the fibrillar layer, mannoprotein, β-glucan, β-glucan-chitin, and mannoprotein (She et al. 2016). The many components of the fungal cell wall collaborate synergistically to maintain cellular integrity and prevent the infiltration of extracellular substances into the cell. *Candida albicans* possesses a plasma membrane consisting of ergosterol, a fungal sterol layer that plays an important part in modulating permeability and regulating the fluidity of membranes (Rubio et al. 2020). The complex structure of the cell wall and plasma membrane in *Candida albicans* might be the factor that contributed to the incapacity of endophytic bacteria's secondary metabolites in inhibiting the growth of *Candida albicans* in this study.

In a study conducted by Aprianti et al. (2019), the antifungal activity of red algae (*Eucheuma spinosum*) extract was examined against *Candida abicans* through phytochemical tests. Despite the presence of alkanoids, flavonoids, steroids, and triterpenoids in red algae, which have been shown to act as antifungals, no inhibitory effect was shown when these compounds were tested against *Candida albicans*. In contrast, a study conducted by Rachman & Sari (2020) proved that a total of 12 bacterial isolates derived from *Lannea coromandelica* were able to inhibit the growth of *Candida albicans*, with the largest inhibition zone diameter measured at 20.75 mm. In addition, a further investigation involved the culture of endophytic bacteria derived from plants of the *Zingeberaceae* family. These bacteria were tested against *Candida albicans*, which led to the observation of an inhibition zone with the largest diameter of 23.7 mm (Nursalwa et al. 2018).

The pathogenicity of *Candida* infections is attributed to various virulence factors, including adhesins, virulence enzymes, and the morphological transition from yeast to hyphae. These characteristics are typically observed in *Candida* infections. In addition, infections caused by *Candida* spp. are distinguished by the formation of biofilms. Biofilms are structures that are embedded within the extracellular matrix (ECM) and have a complex three-dimensional architecture consisting primarily of yeast-shaped cells and hyphal cells with significant heterogeneity (Pierce et al. 2013). The formation of a biofilm starts with the attachment of fungal cells to the surface of the substrate. The biofilm then undergoes a series of stages, including proliferation, maturation, and expansion, which eventually result in the completion of the biofilm development. This cycle can repeat itself, leading to the further extension of the fungal population (Fanning & Mitchell 2012). Biofilm cells possess notable advantages in terms of survival compared to free-living cells since they exhibit enhanced resistance to various antimycotic treatments. The enhanced resistance of *Candida* biofilms to antimycotic drugs and their ability to counter immune defenses, along with their role as a persistent infection reservoir, have substantial clinical implications. The prevalence of biofilms significantly increases the morbidity and mortality associated with *Candida albicans* (Pierce et al. 2013). The formation of biofilms is widely recognized as a prominent virulence factor of *Candida albicans*. It is conceivable to suggest that endophytic bacterial isolates without evident antifungal activity may be incapable of limiting the growth of *Candida albicans*.

In another perspective, it is worth considering that there was a possible factor that could have influenced the results of this study. In a study conducted by Mahulette (2022), the researcher examined endophytic bacteria that were obtained from miana plants. However, the current research re-cultured nine endophytic bacterial isolates without incorporating any additional miana plant filtrate. Host plants and environmental factors play an important role in the formation of secondary metabolites by endophytic bacteria (Wu et al. 2021). In this study, the secondary metabolites produced by endophytic bacterial isolates might have decreased in quantity and quality. The absence of host plant activity, which could help the secretion of secondary metabolites, was a potential cause of this condition. An additional plausible factor was the environmental differences that existed between the host plant and the growth medium. A study conducted by Yunita et al. (2022) isolated endophytic bacteria obtained from *Myristica fragrans*. The results showed that the addition of host plant filtrate to the bacterial isolation process led to a higher total bacterial population, as the plant filtrate contains the growth factors of the endophytic

bacteria.

In the three repetitions of this study, ketoconazole demonstrated a high capacity to inhibit the growth of *Candida albicans*, as evidenced by inhibition zone diameters of 29.5 mm, 26 mm, and 30 mm. Ketoconazole acts as an antifungal agent by blocking the synthesis of ergosterol, thereby weakening the structure and function of the fungal cell membrane (Sinawe & Casadesus 2023). In a similar examination, Kalsum & Ayu (2019) used ketoconazole as a positive control in a test of *Candida albicans*. Their findings revealed inhibition zones with an average diameter of 27.1 mm, signifying a very strong inhibitory effect. As demonstrated by these results, ketoconazole is one of the antifungal drugs that remains sensitive to *Candida albicans*. However, taking into account the potential side effects, ketoconazole should only be administered when absolutely necessary and in accordance with the dosage prescribed by a physician.

Strength and limitations

Miana plants are frequently used as an alternative medicine throughout Indonesia, including the region of Maluku. *Candida albicans* had never been tested against endophytic bacteria derived from Miana plants (*Coleus scutellarioides*). Therefore, the results of this study are important for the development of antifungals using endophytic bacteria derived from miana plants. However, the results might be influenced by the fact that we did not add miana plant filtrate to the growth medium. The addition of host plant filtrate would affect the content of secondary metabolites produced by the endophytic bacteria.

CONCLUSION

Endophytic bacteria derived from miana plants (*Coleus scutellarioides* (L.) Benth.) lack the ability to inhibit the growth of *Candida albicans*. This study implies that ketoconazole remains effective in overcoming infections caused by *Candida albicans*. It is recommended for healthcare professionals who have prescriptive authority to use caution while administering antifungal medications to mitigate the risk of developing fungal resistance.

Acknowledgment

The authors thank Universitas Pattimura, as well as the laboratory assistants of the Maluku Provincial Health Laboratory and Medical Device Calibration Center, Ambon, Indonesia, for their valuable assistance during the research process.

Conflict of interest

None.

Ethical consideration

The Department of Medical Education, Faculty of Medicine, Universitas Pattimura, Ambon, Indonesia, granted ethical clearance for this study with reference No. 173/FK-KOM.ETIK/VIII/2022 on 22/12/2022.

Funding disclosure

None.

Author contribution

All authors contributed to the research process, data analysis, interpretation of results, and drafting of the article. In detail, MY contributed to the research conception and design, critical revision of the article for important intellectual content, and final approval of the article. RML contributed to the analysis and interpretation of the data, as well as the collection and assembly of the data. Lastly, RT contributed to the critical revision of the article for important intellectual content and final approval of the article.

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Original Research Report

DEVELOPMENT OF FORMULAE TO DETERMINE LIVING STATURE USING HANDPRINT ANTHROPOMETRY OF TAGALOG PEOPLE IN THE PHILIPPINES

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ABSTRACT

Forensic science plays a crucial role in the pursuit of justice, particularly through the identification of physical evidence found at crime scenes, such as human fingerprints and handprints. This study aimed to develop formulae for determining living stature using the handprint anthropometry of Tagalog people, an indigenous ethnic group in the Philippines. A total of 360 Tagalog volunteers, comprising 180 men and 180 women, were recruited. This study excluded subjects who had finger and hand-related diseases, injuries, or were under the age of 18 years. The materials used were a stadiometer for height measurement, a digital vernier caliper for handprint measurements, and a handprint kit to collect handprints. Five length measurements were collected for each handprint. The length measurement spanned the distance from the middle wrist crease to the tips of each of the five fingers. The data were analyzed statistically using regression analysis ($p<0.05$) in IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, N.Y., USA). The analysis results produced equations for determining stature using all the length measurements of the handprints. The study involved the calculation of correlation coefficients (r values) and standard deviations using the stature and handprint lengths of individuals of both genders. The results were presented in the form of tables and figures. The study concluded with the development of regression equations that may be utilized for determining stature based on various handprint length measurements of the Tagalog people. This study represents the first-ever anthropological study conducted on the Philippine Tagalog population within the scope of this research subject matter. The formulae can be applied to actual crime scenes for the purpose of personal identification.

Keywords: Forensic science; living stature; handprint; Tagalog people; medicine

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Highlights:
1. This is the first-ever anthropological study on Tagalog people in the Philippines that has established formulae for determining stature using handprint length measurements.
2. This study has generated formulae that are applicable for personal identification purposes within real crime scenes.

INTRODUCTION

The pursuit of justice, particularly in criminal cases, is a complex and multifaceted process that cannot be achieved through a single act. Resolving the cases within an hour, as often depicted in movies, can be a cumbersome task (Mishra 2020). Forensic science represents the intersection of science and law. The field of forensic science focuses on the examination and analysis of physical evidence found at crime scenes. This evidence may appear in either visible or latent forms, viz., impression evidence, which includes fingerprints, handprints, and footprints (Abderrahmane et al. 2021, Asadujjaman et al. 2021). However, other sorts of impression evidence may be helpful in a forensic investigation. Tool marks and tire marks are some other examples of impression evidence (Sharma et al. 2019, Usman et al. 2019, Sørensen et al. 2020). Different types of evidence may also be present at crime scenes, such as trace evidence. This type of evidence includes, but is not limited

to, hair, fiber, bloodstains, and handwriting (Stewart 2017, Singh 2020, Zhang et al. 2022). In a separate study carried out by (Kumar & Khaira 2018), another form of evidence was examined. The research revealed that charred documents found at crime scenes may help establish a link between criminal activities and the criminals involved in those crimes. Invisible or latent impressions can be visualized by applying various chemical powders for further analysis (Singh 2020).

In forensic examinations, the required vital factors for personal identification are the determination of stature, gender, age, and race. These factors are commonly referred to as the "Big Four" (Ubelaker & Khosrowshahi 2019, Dembosky et al. 2019). The inclusion of stature is essential in the construction of a biological profile. Assessment of stature based on different body parts and their impressions is an area of interest to forensic experts, anthropologists, and anatomists. Stature determination can be performed through several anatomical measurements, such as hand, handprint, foot, footprint, finger, fingerprint, orbital cavity, and external ear morphometry. This is feasible since a strong correlation exists between stature and human body parts (Srijith 2019; Antunes et al. 2021).

Anthropometry is a scientific technique used to measure human body parts or their impressions and to understand human physical variation for personal identification. Anthropometric studies are conducted to investigate the evolutionary significance of differences in body proportion between populations whose ancestors lived in different environments (Hemy et al. 2013). This study aimed to examine the relationship between living stature and handprint anthropometry among the Tagalog population in the Philippines and to develop formulae for stature estimation using handprints of the study population for forensic application. It is worth noting that this study represents the first-ever anthropological study of Tagalog people in the Philippines regarding this research subject matter.

MATERIALS AND METHODS

The sample collection was conducted in the Philippines, while the analysis was performed at Management and Science University (MSU), Shah Alam, Malaysia. The ethical clearance for this study was granted by the University Ethics Committee of Management and Science University with certificate No. MSU-RMC-02/FR01/05/L2/007 on 28/2/2020. This study recruited 360 Tagalog volunteers, consisting of 180 males and 180 females, who consented to be participants (Moorthy et al. 2014). All participants were born and resided in the Philippines, and their ages ranged between 18 and 55 years old. Subjects with any hand-related disease, physical impairment, injury, or disorder were excluded from the study. This study used several materials, including a portable stadiometer for height measurements, a fingerprint kit for handprint collection, a digital vernier caliper for handprint length measurements, writing equipment, and A4-size white papers.

The stature of all participants was recorded in cm using a body meter (Model 208, SECA, Germany) from the crown of the head to the sole touching the floor. The measurements were repeated until concordant values were achieved (Moorthy et al. 2014). The Grade "A" fingerprint slab ink (Tritech Forensics, USA) was smeared uniformly on a clean glass plate using a roller. A clean hand was placed on the inked plate with mild pressure. Afterwards, the inked hand was then lifted slowly and impressed on an A4-size white paper, resulting in the transfer of the handprint onto the paper. This process was repeated for all participants. A digital caliper with a length of 300 mm was utilized to get five measurements of length for each handprint. The length was measured from the baseline of the handprint to the tips of each finger, as shown in Figure 1. The measurements covered the distance between the middle wrist crease (LHP) and the tips of all fingers, viz., thumb (T), index finger (I), middle finger (M), ring finger (R), and little finger (L).

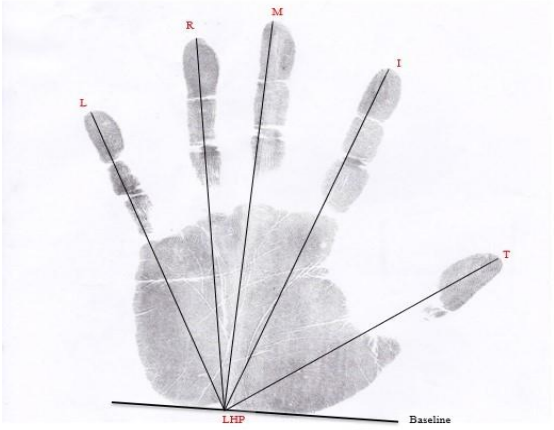

Figure 1. A print showing the various lengths of a participant's left handprint (Asadujjaman et al. 2021).

In order to avoid potential interobserver variability, Kaifi et al. (2019) recommended that all measurements be conducted by a single author (INLD). The data were analyzed statistically using regression analysis in IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk,

N.Y., USA). This analysis aimed to develop formulae that could be utilized for determining stature based on the lengths of handprints in both male and female samples. The threshold of statistical significance was set at $p<0.05$. The participants' stature measurements were organized in a tabular format, displaying the mean values and standard deviations (SD) to illustrate variation between sexes. The standard error of estimate (SEE) served as a measure to assess the accuracy of predictions, which was achieved when the values were between 2 and 6 cm (Moorthy et al. 2019). The presentation of the variance in stature by sex was depicted in graphical form. Similarly, the handprint length measurements from males and females were presented in different tables, along with the corresponding standard deviations. The developed formulae for determining stature based on handprints for males and females were also presented in tabular form. Additionally, the correlation coefficients (r values) that establish the relationship between stature and handprint lengths were calculated. When the r value exhibited a positive value, it indicated that the variables were correlated. The results were presented in the form of tables and figures.

RESULTS

Table 1 displays the recorded stature measurements for both male and female participants within the study samples. The mean stature of the male participants was found to be 163.04 cm, with a variation in height ranging from 147 cm to 184 cm. The mean stature of the female participants was found to be 151.57 cm, with a range of heights spanning from 135 cm to 163 cm. This study observed that the mean stature of males was higher than that of females, as determined by the standard deviation (SD). Figure 2 demonstrates the stature variation among the male and female participants.

Table 1. Stature measurements (cm) of the male and female Tagalog participants.

	Male (n=180)	Female (n=180)
Range	147-184	135-163
Mean	163.04	151.57
SD	6.81	5.16

Notes: n=Sample size; SD=Standard deviation.

Table 2 shows the five lengths on left handprints and the five lengths on right handprints among the male Tagalog participants. The mean handprint lengths with the middle finger were found to be longer, while the handprint lengths with the thumbs were shorter on both sides. In the left handprint, the length between the middle wrist crease and the middle finger (HPM) was the longest, followed by the lengths between the middle wrist crease and the ring finger (HPR), the index finger (HPI), the little finger (HPL), and lastly the thumb (HPT). Whereas, in the right handprint, the length between the middle wrist crease and the middle finger was the longest (HPM), followed by the lengths between the middle wrist crease and the index finger (HPI), the ring finger (HPR), the little finger (HPL), and the thumb (HPT), respectively. Interestingly, the result showed that the left handprint lengths and right handprint lengths were not similar, indicating variation in lengths, known as "bilateral asymmetry". The standard deviation values were very low compared to the height measurement values.

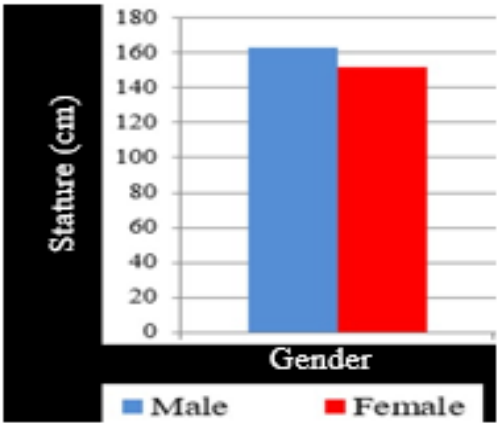

Figure 2. Stature variation by male and female sexes among the Tagalog participants.

Table 2. Handprint measurements (cm) among the male Tagalog participants.

Measurement	Left handprint			Right handprint		
	Range	Mean	SD	Range	Mean	SD
HPT	9.51-13.94	11.76	0.86	10.24-13.95	11.90	0.73
HPI	13.16-18.99	16.07	1.00	14.10-18.92	16.23	0.87
HPM	14.64-20.52	17.10	1.04	14.30-20.16	17.09	0.95
HPR	14.12-19.67	16.27	0.98	14.02-19.71	16.12	0.90
HPL	12.16-15.91	13.93	0.89	12.09-16.57	13.84	0.87

Notes: Handprint length is the distance between the middle wrist crease and the thumb (HPT), the index finger (HPI), the middle finger (HPM), the ring finger (HPR), and the little finger (HPL). SD=Standard deviation.

Table 3 presents the five lengths on left handprints and five lengths on right handprints among the female Tagalog participants. All handprint lengths of the male participants were comparatively longer than the female participants' handprint length measurements. As similarly observed in the male Tagalog participants, the mean handprint lengths measured from the middle finger (HPM) were

found to be longer. The handprint lengths measured from the thumb (HPT) on both the left and right sides were also shorter among the female participants. In the left handprint, the handprint measured from the middle finger (HPM) was the longest, followed by the lengths measured from the ring finger (HPR), the index finger (HPI), the little finger (HPL), and lastly the thumb (HPT). In the right handprint, the longest handprint was measured from the middle finger, followed by the index finger (HPI), the ring finger (HPR), the little finger (HPL), and the thumb (HPT). Similar to the measurement results among the female participants, the left and right handprint lengths among the female participants showed variation and bilateral asymmetry but were not significant. The standard deviation values were very low compared to the height measurement values.

Table 3. Handprint measurements (cm) among the female Tagalog participants.

Measurement	Left handprint			Right handprint		
	Range	Mean	SD	Range	Mean	SD
HPT	9.06-12.93	10.54	0.71	8.17-12.91	10.60	0.77
HPI	12.22-17.94	14.75	0.88	12.32-17.61	14.76	0.92
HPM	13.62-17.85	15.62	0.89	13.10-17.99	15.53	0.95
HPR	13.06-16.92	14.77	0.90	12.40-16.92	14.73	0.97
HPL	10.08-15.83	12.56	0.94	10.41-16.69	12.48	0.89

Notes: Handprint length is the distance between the middle wrist crease and the thumb (HPT), the index finger (HPI), the middle finger (HPM), the ring finger (HPR), and the little finger (HPL). SD=Standard deviation.

The important finding in the investigation was that the declining order of the male and female participants' handprint lengths reflects similarity on both the right-hand and left-hand sides of the study population. The presence of bilateral asymmetry in the handprint lengths of the male and female participants was not so significant, as shown in Figure 3. Hence, the left and right handprint lengths were combined as one side (i.e., HPT, HPI, HPM, HPR, and HPL). Five regression formulae were developed to determine stature using five lengths for male and female stature, as shown in Table 4 and Table 5.

Table 4. Regression formulae to determine stature (cm) using handprint length (cm) measurements of male Tagalogs.

Regression formulae	R	R^2	SEE
S=105.442+4.870HPT	0.52	0.27	5.85
S=83.094+4.951HPI	0.65	0.42	5.21
S=83.200+4.670HPM	0.66	0.43	5.16
S=87.475+4.665HPR	0.60	0.36	5.46
S=99.747+4.559HPL	0.55	0.31	5.69

Notes: S=Stature. Handprint length is the distance between the middle wrist crease and the thumb (HPT), the index finger (HPI), the middle finger (HPM), the ring finger (HPR), and the little finger (HPL). SD=Standard deviation. SEE=Standard error of estimate, ($p<0.001$).

The Pearson correlation coefficient (r) quantifies the extent of association between the variables of height and handprint length. All observed r values demonstrated a strong positive correlation. The r values were positive and statistically significant (<0.001).

Table 5. Regression formulae to determine stature (cm) from handprint length (cm) measurements of female Tagalogs.

Regression formulae	R	R^2	SEE
S=101.008+4.785HPT	0.59	0.35	4.16
S=89.625+4.199HPI	0.70	0.48	3.72
S=87.523+4.114HPM	0.69	0.48	3.75
S=94.035+3.901HPR	0.66	0.44	3.87
S=111.654+3.188HPL	0.51	0.26	4.46

Notes: S=Stature. Handprint length is the distance between the middle wrist crease and the thumb (HPT), the index finger (HPI), the middle finger (HPM), the ring finger (HPR), and the little finger (HPL). SD=Standard deviation. SEE=Standard error of estimate, ($p<0.001$).

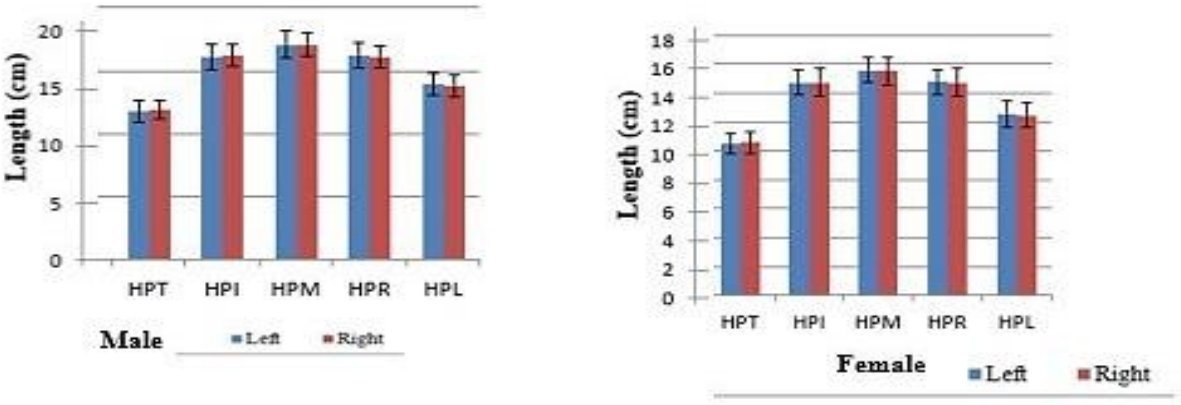

Figure 3. Various handprint lengths for both sexes as well as left-hand and right-hand sides.

The correlation coefficient (r) values were higher among the female samples (0.51–0.70) when compared with the male samples (0.52–0.66). The female participants exhibited a higher coefficient of determination (R^2), indicating higher predictive accuracy compared to the male participants. All measurements were positive and statistically significant for the stature estimation. The observed values in this study were small, indicating that the estimation of stature was achieved with enhanced accuracy.

DISCUSSION

The Philippines is a country characterized by its diverse ethnic composition, comprising around 120 distinct ethnic groups. Each of these ethnic groups has its own language and unique traditions. The Tagalog people are an indigenous ethnic group, with Tagalog being recognized as one of the two official languages in the Philippines (Talabis et al. 2013). In the current demography of the Philippines, it is observed that males constitute 50.1% of the total population, while females account for 49.9%. There is a considerable amount of research available on the topic of determining stature from footprints and shoeprints. On the contrary, studies on palm prints or handprints are rare due to a limited knowledge of the subject matter, particularly in the Philippines. In our prior experiences of crime scene investigation, it was frequently found that the perpetrators of the crimes would leave latent handprints at burglary scenes, whereas visibly stained prints were left behind at bloody murder scenes.

Crime scene professionals face challenges when attempting to estimate the stature of culprits using unidentified handprints found at the scenes of burglary, homicide, and sexual assault cases. In such cases, it is mandatory to determine the stature of the crime offenders based on the handprints found at the respective crime scenes. A practical alternative approach involves the development of novel formulae. Consequently, this work has undertaken the task of developing regression formulae for determining the stature based on the handprints of the Tagalog population. The formulae can be utilized to either include or exclude individuals under suspicion whenever they are brought to police stations (Vernon et al. 2020). The current state of research on handprints is notably scarce due to a reluctance among individuals to share their fingerprints or handprints with others, even when they are in a familial or friendly relationship.

Table 6 compares the stature measurements between the present study population and other populations, highlighting the observed ethnic variations. The data presented in the table demonstrates a consistent pattern of males having a larger average stature than females across all populations. This observation underlines the natural variation between the sexes within the analyzed samples. The comparison of the studies revealed that Western Australian males and Iraqi males have comparable stature (Hemy et al. 2013, Farhan et al. 2023). Western Australian males were found to have the tallest height (178.47 cm), while Iraqi males ranked second in terms of stature (177.71 cm). This was followed by Punjabis in Pakistan (173.42), UPites in Uttar Pradesh, India (173.20), Chinese Malaysians (171.50 cm), Saudi Arabians (170.80 cm), Malays in West Malaysia (168.70 cm), Ibans in East Malaysia (164.80 cm), Minangs in Indonesia (163.85 cm), and lastly Tagalogs (163.40 cm) of the present study, Kagay-anons (162.06 cm), and Visayans in the Philippines (161.57 cm) (Asif 2021, Elelemi et al. 2021, Mishra & Vardaini 2022).

Table 6. Comparison of Tagalog stature (cm) with other ethnic groups showing the ethnicity variation.

Ethnic Groups	Region/Country	Stature	
		Male	Female
Malays	West Malaysia	168.70	156.30
Chinese	West Malaysia	171.50	158.20
UPites	Uttar Pradesh, India	173.20	163.20
Saudi	Saudi Arabia	170.80	159.60
Punjabis	Pakistan	173.42	162.09
Minangs	Indonesia	163.85	152.39
Ibans	East Malaysia	164.80	153.50
Kagay-anons	Philippines	162.06	151.35
Visayans	Philippines	161.57	150.84
Western Australians	Australia	178.47	163.67
Iraqis	Iraq	177.71	161.79
Tagalogs (present study)	Philippines	163.40	151.57

Numerous studies have reported a significant correlation between the length of the middle finger and an individual's stature. A study conducted by Asha et al. (2012) found a strong correlation between the length of the middle finger and the stature of Indian adults. Similar findings were revealed in several studies conducted in other countries. One of which is a study carried out by Sanli et al. (2005) that found a moderate correlation between the length of the middle finger and the stature of Turkish individuals. However, it is important to note that these correlations may not universally apply to all populations. Additional variables, such as genetics, nutrition, and environmental factors, play a significant influence

on an individual's stature (Grasgruber et al. 2014). Forensic and anthropological studies on human stature, which involve diverse multicultural and multiethnic groups across different countries, play a pivotal role in establishing comprehensive databases that may be effectively used in actual forensic investigations.

Strength and limitations

Prior studies have developed formulae to estimate an individual's stature using the measurement of handprint length from a single hand. In this study, the development of formulae was achieved using five length measurements for each handprint in both male and female subjects. In many instances, only partial handprints are found at crime scenes, wherein only one handprint is left, either with the thumb or middle finger, and so on. The developed formulae from this study can be used for determining stature, even when partial handprints are present at crime scenes. However, it is important to acknowledge the limitations of this study. The derived equations are applicable only to the Tagalog population, rather than serving as general equations that can be applied to all populations in the world.

CONCLUSION

The regression formulae derived in this study can determine living stature by making use of handprint anthropometry, thus successfully achieving the research objective. It is recommended that future research attempts to investigate other populations and develop more extensive databases that may be useful in crime scene investigations.

Acknowledgment

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Conflict of interest

None.

Ethical consideration

The University Ethics Committee of Management and Science University, Shah Alam, Malaysia, approved this study with certificate No. MSU-RMC-02/FR01/05/L2/007 on 28/2/2020.

Funding disclosure

None.

Author contribution

TNM designed the study, analyzed the data, and corrected the draft manuscript. INLD collected the samples in the Philippines, analyzed the data, and drafted the manuscript. MDA reviewed, revised, and submitted the manuscript.

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Original Research Report

TRAUMATIC BRAIN INJURY PATIENTS IN THE EMERGENCY UNIT OF A TERTIARY HOSPITAL

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ABSTRACT

Traumatic brain injury (TBI) is a prevalent neurological condition in emergency units. TBI cases are frequently diagnosed with severe conditions. Underdiagnosis is common in mild TBI cases. As a result, physicians have an uncomprehensive understanding of patients' characteristics in their daily practice. This study aimed to discuss the characteristics of trauma patients who were diagnosed with TBIs in an emergency unit. We conducted a retrospective cohort observational study to examine the adult population of TBI patients from April 2022 to March 2023. This study collected several data points, including demographics, clinical characteristics, surgical procedures, and disposition distribution. All variables were compiled and summarized using descriptive statistics and analyzed by calculating frequencies and percentages. During the period of this study, 483 trauma patients were admitted to the emergency unit. A total of 361 (74.7%) of these cases were identified as TBIs. Most of the traumatic brain injuries occurred in men (75.6%), with the predominant age range being 18–22 years (22.4%). Mild TBI cases (74.5%) were the most prevalent, followed by moderate (20.2%) and severe (5.3%) TBI cases. The average score from the Glasgow Coma Scale assessment was 15. Most patients (80.9%) had no hypotension, while a greater number of patients (98.1%) did not exhibit hypoxia as a comorbidity. The Injury Severity Score revealed that the highest percentage of TBIs was categorized as minor (62.3%). It was found that 53.5% of the patients were discharged, while 36% of the patients were hospitalized. However, seven patients (1.9%) passed away in the emergency unit, and 30 patients (8.3%) opted to be discharged against medical advice. Only 21 patients (5.8%) received neurosurgical management. As the most prevalent trauma, TBI necessitates careful management to handle the implications of clinical decision-making. It is crucial to investigate the underlying risk of mortality in TBI cases because the majority of patients do not require neurosurgical intervention.

Keywords: Emergency unit; health system; patient characteristics; traumatic brain injury

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Highlights:
1. The main focus of this study was to underscore the importance of exploring the characteristics of traumatic brain injury (TBI) patients, which have not been extensively investigated, to help establish effective clinical procedures and decisions.
2. Three out of every four trauma patients in the emergency unit had mild TBIs, and those who did not survive showed a significant trend of rapid deterioration.
3. It is crucial to consistently conduct a thorough and comprehensive head-to-toe examination for all trauma patients.

INTRODUCTION

Traumatic brain injury (TBI) is a neurological emergency that has become a global public health problem. Around 50–60 million people experience TBI annually (Steyerberg et al. 2019, Maas et al. 2022). A study in California, USA, found that the incidence of TBI in emergency units increased by over 50% during the last ten years. This condition causes high rates of disability and mortality worldwide, especially in low- and middle-income countries. TBI burdens healthcare systems and

economies through lost productivity, high healthcare costs, and long-term care (Watanitanon et al. 2018. Hsia et al. 2018).

TBI is a heterogeneous condition in terms of causes, severity, and outcomes. Regarding severity, TBI is categorized into three clinical types: mild, moderate, and severe. Many clinicians focus on moderate and severe TBIs, as they are more serious than mild TBIs. This situation makes mild TBIs under-diagnosed. TBI patients, including those with mild TBIs, may experience sequelae after discharge. Consequently, their functional condition will not be the same as before the injury (Dewan et al. 2019, Pozzato et al. 2020, Hagos et al. 2022).

Due to the lack of data collection on patient characteristics, clinicians will not be able to understand TBI comprehensively and will hesitate to make clinical decisions in an emergency setting. In addition, understanding the characteristics of TBI is the key to determining management and assessing patient prognosis, especially in emergency units (Maas et al. 2022). Therefore, this article aims to provide an overview of the characteristics of TBI patients in the emergency unit.

MATERIALS AND METHODS

This observational study used a retrospective cohort design to examine trauma patients who received treatments at the emergency unit of Rumah Sakit Umum Pusat Nasional Dr. Cipto Mangunkusumo, a tertiary teaching hospital located in Jakarta, Indonesia. The study was conducted from April 2022 to March 2023. We collected data from patient medical records. The inclusion criteria were patients aged 18 years or older who were diagnosed with TBI upon admission to the emergency unit. The exclusion criteria were patients with incomplete data that could not fulfill the necessary variables (Ghandour et al. 2022).

Several data points were collected during the course of this study, such as demographic information, clinical characteristics, surgical procedures, and disposition distribution. One of the clinical variables examined in this study was TBI severity, which was assessed using the Glasgow Coma Scale score. A total score of 3–8 was indicative of a severe TBI, while score ranges of 9–12 and 13–15 indicated a moderate and mild TBI, respectively. Other variables were also documented in this study, including the classification of systolic blood pressure of <110 mmHg as hypotension and the classification of oxygen saturation of ≤90% as hypoxia (Gaitanidis et al. 2021, Eom et al. 2021).

All data were analyzed by calculating frequencies and percentages using IBM SPSS Statistics version 25.0. The analysis results were presented descriptively in the form of tables and figures (Steyerberg et al. 2019). The Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia - Rumah Sakit Umum Pusat Nasional Dr. Cipto Mangunkusumo, Jakarta, Indonesia, granted approval for this study with registration No. KET-629/UN2.F1/ETIK/PPM.00.02/21 on 21/06/2021.

RESULTS

As shown in Table 1, there were 483 trauma patients admitted to the emergency unit during the study period. Approximately 361 (74.7%) of the patient population presented with TBIs, with nearly three cases in every four patients. Among those TBI patients, males (75.6%) were three times more common than females (24.4%). The histogram shows that the patients' median age was 34, with a range spanning from 18 to 84 years (Figure 1). This median indicated a prevalence of TBIs in the productive age group.

Table 1. Characteristics of the TBI Patients (n=361).

Variables	Classification	Number	%
Sexes	Male	273	75.6
	Female	88	24.4
TBI severity	Mild	269	74.5
	Moderate	73	20.2
	Severe	19	5.3
GCS score		15 (3–15)*	
Hypotension	Yes	69	19.1
	No	292	80.9
Hypoxia	Yes	7	1.9
	No	354	98.1
Injury Severity Score	Minor (1–8)	225	62.3
	Moderate (9–15)	87	24.1
	Serious (16–24)	31	8.6
	Severe (25–49)	16	4.4
	Critical (50–74)	0	0
	Unsurvivable (75)	2	0.6
Neurosurgical management	Yes	21	5.8
	No	340	94.2
Outcome disposition	Discharged	193	53.5
	Discharged against medical advice	30	8.3
	Hospitalized	131	36.3
	Death	7	1.9

Notes: *Median (minimum-maximum). Traumatic brain injury (TBI). Glasgow Coma Scale (GCS).

According to the level of severity, this study found that mild TBIs were the most common, with approximately 296 patients (74.5%). This was followed by moderate TBIs in 73 patients (20.2%) and severe TBIs in 19 patients (5.3%). In the measurement of the patients' level of consciousness using the Glasgow Coma Scale, the average score was 15.

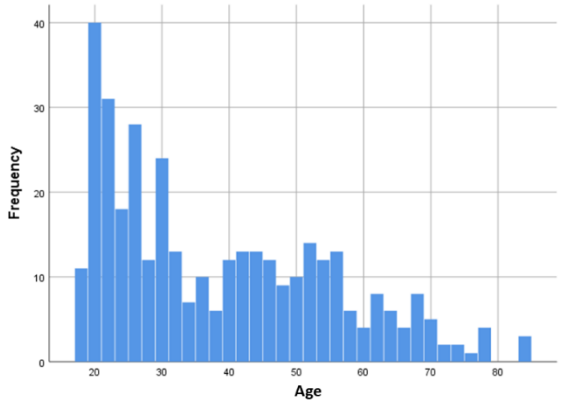

Figure 1. Age distribution of the TBI patients.

Regarding the severity and complications of TBI, nearly one-fifth of the patients had hypotension (19.1%). Furthermore, most patients did not experience hypoxia (98.1%). According to the assessment using the Injury Severity Score (ISS), the majority of TBI patients were classified as having minor severity (62.3%), with the subsequent highest classification being moderate severity (24.1%).

Within the population of TBI patients, there was a deficient proportion of TBI patients who were managed surgically (5.8%). Regarding disposition, a total of 193 patients (53.5%) were discharged. A smaller proportion of patients (36.3%), with a total of 131 individuals, were admitted to the hospital. It is worth noting that seven patients (1.9%) died in the emergency unit and 30 patients (8.3%) were discharged against medical advice (DAMA). Among seven deceased individuals in the emergency unit, three were categorized as suffering from severe TBIs, three were classified as having moderate TBIs, and one was identified as having a mild TBI.

DISCUSSION

This study can provide novel descriptive data regarding TBIs in real-world situations, especially in low- and middle-income countries. In the span of one year, a total of 483 trauma patients were admitted to the emergency unit, out of which 361 individuals were diagnosed with TBIs. This observation illustrated that on a daily basis, the emergency unit received approximately one to two trauma patients. Three out of four trauma patients experienced TBIs. Most patients in our research were under the age of 60. This observation aligned with other studies that had identified a high incidence of TBIs within younger adult populations (Putra et al. 2021, Magalhães et al. 2022). This was also consistent with the findings of a study conducted at a referral hospital in Uganda. The study revealed that the majority (68%) of TBI patients fell within the age range of 18–50 years (Luggya et al. 2022). However, it should be noted that different studies had presented data suggesting that age variations might arise due to differences in inclusion criteria, such as population coverage, mechanism of injury, or other criteria as defined by the respective studies (Peeters et al. 2015, Skaansar et al. 2020, Brazinova et al. 2021).

The results of our study indicated a higher prevalence of TBIs among males (75.6%). The finding was supported by multicenter epidemiological studies conducted in European countries and Korea. According to one of the previous multicenter studies, men had a higher likelihood of being employed in occupations that included a greater risk of injury or hazardous behaviors, hence making them more vulnerable to sustaining injuries (Peeters et al. 2015, Eom et al. 2021). The analysis of demographic data revealed that TBIs disproportionately impacted males within the most productive age group. This condition significantly affected their socioeconomic status, as they often take on the role of the primary breadwinner in their families. The family's financial condition would be disrupted due to their inability to earn income (Zia et al. 2019, Lee et al. 2021, Luggya et al. 2022). Traumatic brain injuries (TBIs) have been deemed a major contributor to the economic burden imposed on the healthcare system, in addition to patients, their families, communities, and the country. It has been estimated that TBIs incur an annual economic cost of approximately US$400 billion on a global scale (Maas et al. 2017, Howe et al. 2022).

In this study, the majority of the patients experienced mild TBIs. These findings were similar to those observed in research conducted across 18 European countries through the Collaborative European NeuroTrauma Effectiveness Research in Traumatic Brain Injury (CENTER-TBI) (Steyerberg et al. 2019, Hagos et al. 2022). A study conducted in Africa demonstrated a positive correlation between the severity of TBI and mortality rates. The study found that the distribution of severity levels was 25% for severe cases, 8.0% for moderate cases, and 2.0% for mild cases (Hagos et al. 2022). The mortality rates associated with mild TBIs are low. Nevertheless, it is noteworthy that around a quarter of patients in the emergency unit and half of hospitalized patients diagnosed with mild TBIs do not fully recover within six months. This illness creates a significant social burden for healthcare services and affects individuals' functional recovery and quality of life (Dewan et al. 2019, Gao et al. 2020, MagalhãesS et al. 2022).

Hypotension and hypoxia are identified as risk factors that contribute to the increased mortality associated with TBIs. Both disorders have the potential to result in secondary brain injury (Aninditha & Wirataman 2017, Spaite et al. 2017, Rauch et al. 2021). The findings of our study showed that most of the patients did not exhibit symptoms of hypotension or hypoxia. This condition was related to the disposition of our patient population, wherein the mortality rate was only 1.9% within the emergency unit. According to a study conducted in Boston, USA, it was shown that there is an association between systolic blood pressure levels of ≥110 mmHg and a decrease in hospital mortality rates among adult patients with TBIs, including individuals between the ages of 50 and 69. According to the Brain Trauma Foundation guidelines, individuals aged 50–69 years should maintain a systolic blood pressure of at least 100 mmHg, while those aged 15–49 and >70 years should aim for a systolic blood pressure of at least 110 mmHg (Carney et al. 2017, Gaitanidis et al. 2021). Hypoxic conditions have the potential to induce irreversible brain injuries. The brain injuries may persist until the onset of cerebral anoxia (Ankita et al. 2015, Zairinal 2022). However, the results of our study were incomplete in terms of providing a comprehensive description of the patients' systolic blood pressure and oxygen saturation due to the absence of clear documentation pertaining to these variables before or after resuscitation.

According to the Injury Severity Score assessment results, most patients in this study had a score of <16. This observation might be attributed to the high prevalence of individuals receiving outpatient care and presenting with mild TBIs. Nevertheless, an Injury Severity Score assessment result of ≥16 cannot be used as a reliable indicator for identifying patients who are at a high risk of mortality. The assessment of mortality risk should be conducted on an individual basis with a clinical evaluation (Girshausen et al. 2022, Colnaric et al. 2022, Dehouche 2022).

The present study observed a relatively low proportion of patients who required neurosurgical management. The large number of mild TBI patients in this study indicated that there was almost certainly no requirement for neurosurgical intervention. Out of a total of 92 patients diagnosed with moderate and severe TBIs, only 19 individuals underwent surgery. The analysis of the data revealed that the precise timing of stabilization, monitoring, and further therapies play a critical role in mitigating morbidity and mortality, hence enhancing patient outcomes (Valle et al. 2022, Zairinal 2022, Picetti et al. 2023)

Through examining the deceased TBI patients, it was observed that there was an evident trend wherein patients with moderate TBIs showed a tendency for rapid deterioration. Either moderate or severe TBIs must be treated aggressively and by involving many resources. These resources include reliable personnel specializing in caring for critical neurological patients, the availability of intensive care unit (ICU) beds, and the implementation of a multimodal neuromonitoring approach. Moreover, it is essential to provide thorough and comprehensive care to trauma patients, ensuring a full examination and treatment from head to toe, particularly in mild TBI cases (Watanitanon et al. 2018).

Strength and limitations

This study has the potential to contribute to the current collection of research as a novel descriptive data study focusing on traumatic brain injuries (TBIs) in emergency settings and real-world situations, specifically in low- and middle-income countries. The present study provides an analysis of the patients' trajectory within the emergency unit, including their admission and discharge, based on the clinical data obtained. In accordance with the analysis, it is crucial to take into account the duration of management for TBIs, as it can affect the later stages of hospitalization and rehabilitation. However, several cases of discharge against medical advice limited the potential of the data to comprehensively describe the clinical profiles of TBI patients. Additionally, this study was unable to obtain additional important clinical data, such as pupil reactivity, patient consciousness, and blood pressure, that were uncertain between pre- and post-resuscitation. Further research is required in order to present more comprehensive characteristics of TBI patients.

CONCLUSION

This study has identified mild traumatic brain injuries (TBIs) as the most prevalent among those within the most productive age group, with a higher prevalence observed in men than women. While a considerable number of patients did not require neurosurgical interventions and were subsequently discharged, it is important to note that several patients died in the emergency unit. Failure to perform a brief neurologic examination during the primary survey of trauma patients may indicate only the tip of the iceberg.

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Conflict of interest

None.

Ethical consideration

This research was ethically approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia - Rumah Sakit Umum Pusat Nasional Dr. Cipto Mangunkusumo, Jakarta, Indonesia, with registration No. KET-629/UN2.F1/ETIK/PPM.00.02/21 on 21/06/2021.

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None.

Author contribution

RAZ contributed to conceptualization, study design, formal analysis, data interpretation, methodology, provision of study materials, funding acquisition, supervision, manuscript writing, and content revision. CNM contributed to data gathering, manuscript writing, and project administration. YR, DNL, AM, and PP contributed to conceptualization, study design, formal analysis, data interpretation, methodology, supervision, manuscript writing, and content revision.

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Original Research Report

ANTIFUNGAL ACTIVITY OF *Selaginella plana* (Desv. ex Poir.) Hieron EXTRACT AGAINST *Candida albicans* IN VITRO

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ABSTRACT

Candidiasis is an opportunistic infection caused by *Candida albicans*. This infection commonly affects the skin, oral mucosa, vagina, and gastrointestinal tract. Excessive use of azole antifungals for treating *Candida albicans* infections can lead to the development of resistance. Therefore, it is necessary to explore alternative treatments using medicinal plants such as *Selaginella plana*, commonly referred to as "*rutu-rutu*" in a local language spoken across Maluku, Indonesia. *Selaginella plana* contains active compounds from various chemical classes, including terpenoids, steroids, flavonoids, and saponins. This study aimed to determine the ability of *Selaginella plana* extract as an antifungal agent against *Candida albicans* by evaluating its inhibitory and antifungal effects. This study used an actual experimental design and broth dilution method. The research methodology involved the extraction of *Selaginella plana* using a solvent of 96% ethanol. The extract was then prepared in various concentrations, i.e., 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. Additionally, ketoconazole and distilled water were included in the experiment for the positive and negative controls, respectively. The results of this study demonstrated that *Selaginella plana* extract inhibited the growth of *Candida albicans* when administered starting at a concentration of 12.5%. However, the antifungal potential of *Selaginella plana* extract that induced cell death was only observed at a concentration of 100%. The fungicidal activity was exclusively identified in the undiluted, pure extract. The inhibitory and cytotoxic effects of *Selaginella plana* on *Candida albicans* cells were attributed to the presence of bioactive compounds in *Selaginella plana*, including flavonoids, tannins, terpenoids, and saponins. These bioactive compounds had the ability to inhibit cell growth by altering membrane permeability, causing mitochondrial dysfunction, and disrupting ergosterol biosynthesis. It can be concluded that *Selaginella plana* extract can act as a fungistatic agent against the proliferation of *Candida albicans*.

Keywords: Antifungal medicine; *Selaginella plana*; *Candida albicans*; infectious disease; medicine

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Highlights:
1. A study on the medical benefits of *Selaginella plana* has significant academic value due to its extensive traditional usage among the Moluccan people as a medicinal remedy, especially for its antifungal properties.
2. The findings of this study will allow further screening to determine the mechanism of action of bioactive compounds in inhibiting the growth of *Candida albicans*.

INTRODUCTION

Fungal infections pose microbial threats to human health. According to a study conducted by Bongomin et al. (2017), fungal infections cause about 1.5 million deaths each year. Mortality rates caused by fungal infections continue to increase despite improvements in social development and health status. *Candida albicans* is the most common fungus responsible for human infections. *Candida albicans* is a normal flora commonly found in the skin, vagina, digestive tract, and respiratory tract. However, it also has the potential to induce opportunistic infections. *Candida albicans* can

invade tissues and organs, affecting about 50% of the world's population (Naglik et al. 2014). One of the surgical infections caused by *Candida albicans* is candidiasis, which can infect the surface of the skin, oral mucosa, and penis. The predominant cause of invasive candidiasis was *Candida albicans*, accounting for 46.3% of cases, followed by *Candida glabrata* (24.4%) and *Candida parapsilosis* (8.1%) (Andes et al. 2016). Candidiasis is commonly referred to as a yeast infection because the causative agent is yeast. Candidiasis frequently manifests as a secondary infection in individuals with weak immune systems (Qadir & Asif 2020).

The global trend in candidiasis prevalence is concerning, as *Candida* species continue to be the main causative agents of invasive fungal infections. On a global scale, the annual prevalence of invasive candidiasis exceeds 750,000 cases (Bongomin et al. 2017). Prevalence data from a study by Pegorie et al. (2017) showed that there were 5,142 candidiasis cases in England. The data also revealed 28,991 cases in Brazil, 38,795 cases in Pakistan, 11,840 cases in Russia, and 4,540 cases in Vietnam. The Mycology Division of the Department of Dermatology and Venereology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, reported candidiasis cases in the period of 2013–2016. According to the report, the prevalence rates of candidiasis were observed to be 6.23% (99 patients) in 2013, 6.08% (77 patients) in 2014, 5.58% (55 patients) in 2015, and 8.97% (67 patients) in 2016 (Puspitasari et al. 2019).

The main classes of antifungal drugs used for the treatment of *Candida* infections include azoles, polyenes, echinocandins, pyrimidines, and allylamines. Although these antifungal drugs have been widely used to treat *Candida albicans* infections, misuse and overuse of these treatments can result in the development of resistance. According to data from the Centers for Disease Control and Prevention (2019), there has been a significant increase in *Candida albicans* resistance to antibiotics, with a total of 34,800 recorded infection cases and 1,700 deaths. This indicates that the efficacy of antifungal drugs in treating infections caused by *Candida albicans* has been limited, which leads people to seek alternative treatments using medicinal plants. Plants produce a variety of secondary metabolites that function as defense compounds against herbivores and microorganisms (Wink 2015). *Selaginella plana* is a medicinal plant commonly used by the Moluccan people in Indonesia. The plant is referred to as "*rutu-rutu*" in a native language spoken in the Maluku region of Indonesia.

Selaginella plana has been used as an alternative medicine in several traditional medicinal practices, including wound healing, postpartum care, management of menstrual disorders, treatment of skin diseases, headache relief, as well as treatment for fever, respiratory tract infections, urethral infections, cirrhosis, cancer, and rheumatism. *Selaginella plana* is known to have a diverse range of bioactivities, including antitumor, antibiotic, antiviral, antioxidant, hypoglycemic, anti-inflammatory, antimalarial, antidiabetic, and anti-aging effects (Xu et al. 2019). In our previous study, we examined the beneficial effects of *Selaginella plana* as an antimicrobial agent capable of inhibiting the growth of *Staphylococcus aureus*. People use *Selaginella plana* as a traditional medicine because it contains secondary metabolites such as alkaloids, flavonoids, and terpenoids (Warella et al. 2020). However, the use of *Selaginella plana* as an antifungal agent has never been reported. Hence, this study aimed to test the potential of *Selaginella plana* extract as an antifungal agent in inhibiting the growth of *Candida albicans*.

MATERIALS AND METHODS

This study was a pure experiment using a randomized post-test-only control group design. The experiment consisted of three repetitions conducted on six experimental groups, along with a positive control and a negative control. An observation of the experimental groups was performed in relation to the application of six treatment concentrations (Parnomo 2021). The fungal specimens were pure cultures of *Candida albicans* ATCC 10231 obtained from the Surabaya Health Laboratory Center, Surabaya, Indonesia (7°16'2.53328"S, 112°45'37.13756"E). The experimental samples included different concentrations of *Selaginella plana* (Desv. ex Poir.) Hieron extract sourced from Kairatu Village, Maluku, Indonesia (3°12′55″S, 128°10′34″E). The identification of *Selaginella plana* plants was conducted at the Plant Conservation Center, located within Purwodadi Botanical Garden in Pasuruan, Indonesia. The objective of this procedure was to match the observed characteristics of the *Selaginella plana* samples with the plant identification key, which follows the binomial nomenclature classification system. This procedure was carried out as it served the purpose of identifying the specific plant species before conducting the experiment. It was helpful in minimizing errors in the collection of samples and mitigating the risk of unexpected contamination with other plant species.

The *Selaginella plana* extraction process was carried out using a maceration method for three 24-hour intervals. The extraction process incorporated 96% ethanol as a solvent. One notable advantage of the maceration method was that it was performed

without heating, allowing thermolabile compounds to be extracted. A total of 5 kg of *Selaginella plana* leaves were washed, cut into small pieces, dried in an oven at 40–60°C, and pulverized into powder. After that, 100 g of powder were added to 1 L of 96% ethanol. The mixture was then left to stand for one night with occasional stirring. The next step was separating the ethanol from the *Selaginella plana* extract using an evaporation flask at 60°C (Septiani et al. 2021).

The fungal rejuvenation process was carried out by obtaining a smear of *Candida albicans* from a pure culture using an inoculation loop. Afterwards, the samples underwent incubation for 72 hours at a temperature of 37°C on Sabouraud dextrose agar. The purpose was to sustain the viability and purity of the organisms, prevent any variation or mutation, and generate a new and fresh culture for successful reproduction (Saigal et al. 2011). *Candida albicans* suspensions were prepared in test tubes using 10 mL of Mueller-Hinton broth medium. The researchers put a smear of the rejuvenated microbial samples using an inoculation loop into the Mueller-Hinton broth medium. Following that, we carried out the vortex process until they achieved homogenous suspensions.

The experiments were conducted using the dilution method, following the guidelines set by the Clinical and Laboratory Standards Institute (2020), to determine the minimum inhibitory concentration and minimum fungicidal concentration (MFC). The application of the dilution method offered an advantage in assessing the efficacy of the compounds in terms of inhibitory and fungicidal properties against *Candida albicans*. In addition, this method enabled the quantitative assessment of microorganisms and facilitated the simultaneous evaluation of multiple concentrations within a single experimental assay. The Mueller-Hinton broth was used as the medium in this study. In this experiment, there were six treatment groups with different extract concentrations, i.e., 100% (P1), 50% (P2), 25% (P3), 12.5% (P4), 6.25% (P5), and 3.125% (P5). Additionally, this experiment included a positive control (K+) and a negative control (K-) that received ketoconazole and distilled water, respectively.

The first step was to obtain a bacterial suspension equivalent to the 0.5 McFarland standard. In each of the treatment groups (P1, P2, P3, P4, P5, and P6), 1 mL of *Selaginella plana* extract at predetermined concentrations was combined with 1 mL of Mueller-Hinton broth. The mixture was put into a vortex mixer to allow homogenization of the solution within the test tubes. Afterwards, the treatment mixture was added to 1 mL of microbial suspension for each of the treatment groups. The procedure was replicated three times. Following a 72-hour incubation period at a temperature of 37°C within an incubator, the treatment groups were observed and compared with both the positive and negative control groups (Fitriana et al. 2020).

The minimum inhibitory concentration was assessed for both the treatment groups and control groups following a 72-hour incubation period. The determination of the minimum inhibitory concentration was conducted by observing small samples and identifying which concentration of the *Selaginella plana* extract could effectively block fungal growth, as visually confirmed by three observers. The visual observations required the identification of any turbidity within the experimental groups. The presence of turbidity indicated that a fungal growth was observed to be positive. In contrast, the absence of turbidity indicated the absence of fungal growth. Subsequently, the minimum inhibitory concentration was established (Kowalska-Krochmal & Dudek-Wicher 2021). In addition, the establishment of the minimal fungicidal concentration involved the observation of a culture on Mueller-Hinton agar, wherein the concentrations of the extract varied. This observation was conducted over a period of 72 hours at a constant temperature of 37°C. The *Candida albicans* colonies were measured using a colony counter. The *Selaginella plana* extract was determined to have fungicidal properties when the number of colonies was below 10. However, its efficacy in killing *Candida albicans* diminished when the colony count exceeded 10 colonies (Balouiri et al. 2016).

RESULTS

This study analyzed the administration of *Selaginella plana* extract using the broth dilution method. In this experimental approach, the researchers employed a sequential dilution process to produce a range of concentrations from the extract. The concentrations obtained were 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. Distilled water served as the negative control, whereas ketoconazole was used as the positive control. Table 1 shows the results of the minimum inhibitory concentration test conducted on the *Selaginella plana* extract.

The experimental findings indicated that the extract derived from *Selaginella plana* had inhibitory effects on *Candida albicans*, with inhibition observed at concentrations of 100% up to 25%. Consequently, a concentration of 25% was determined to be the minimum inhibitory concentration. The group that received ketoconazole as a positive control did not exhibit any turbidity, but

the control group that received distilled water as a negative control displayed turbidity.

Table 1. Minimum inhibitory concentration test results of *Selaginella plana* extract against *Candida albicans*.

Groups	Fungal growth		
	1st replicate	2nd replicate	3rd replicate
100%	-	-	-
50%	-	-	-
25%	-	+	-
12.5%	+	+	+
6.25%	+	+	+
3.125%	+	+	+
Positive control	-	-	-
Negative control	+	+	+

Notes:
(-): Absence of fungal growth.
(+): Presence of fungal growth.

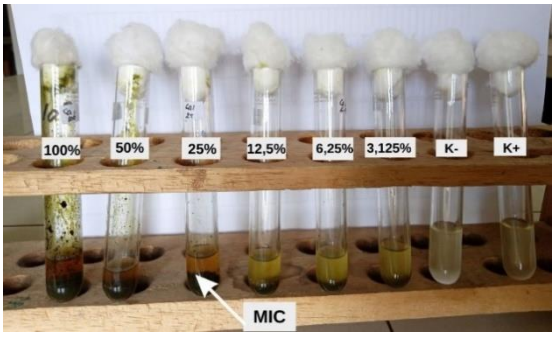

Figure 1. Observation of the minimum inhibitory concentration of *Selaginella plana* extract.

A test was undertaken to determine the minimum inhibitory concentration (MIC) of *Selaginella plana* extract at various concentrations (100%, 50%, 25%, 12.5%, 6.25%, and 3.125%). The experiment involved a comparison with ketoconazole and distilled water as the positive and negative controls, respectively. Figure 1 shows that the minimum inhibitory concentration of *Selaginella plana* extract was determined to be 25%, as indicated by the white arrow.

Table 2 presents the results of the examination on the minimum inhibitory concentration of *Selaginella plana* extract against *Candida albicans*. The results from the experiment indicated that the *Selaginella plana* extract had the ability to inhibit the growth of *Candida albicans* when administered at a concentration of 100%. Figure 2 depicts the proliferation of *Candida albicans* colonies on the petri dishes. Only a single colony of *Candida albicans* was observed within the area where a 100% concentration of *Selaginella plana* was administered. Hence, the presence of less than 10 colonies of *Candida albicans* was interpreted as a negative result.

Tabel 2. Minimum fungicidal concentration test results of *Selaginella plana* extract against *Candida albicans*.

Groups	Minimum fungicidal concentration		
	1st replicate	2nd replicate	3rd replicate
100%	-	-	-
50%	+	+	+
25%	+	+	+
12.5%	+	+	+
6.25%	+	+	+
3.125%	+	+	+
Positive control	-	-	-
Negative control	+	+	+

Notes:
(-): Absence of fungal growth.
(+): Presence of fungal growth.

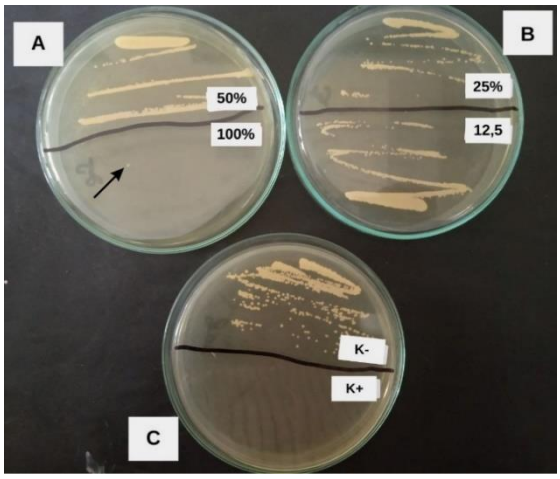

Figure 2. Minimum fungicidal concentration test of *Selaginella plana* extract at concentrations of 50% and 100% (A), 25% and 12.5% (B), and ketoconazole and distilled water (C).

DISCUSSION

Candida albicans is a pathogenic microorganism that frequently causes infections in humans. Many studies have reported the emergence of antifungal medication resistance in *Candida albicans* (Ksiezopolska & Gabaldón 2018). The resistance has been observed to arise as a consequence of the extensive use of antifungal drugs, such as fluconazole and voriconazole, for both prophylactic

and therapeutic purposes. This situation becomes a potential background for the advancement of antifungal drugs derived from natural ingredients, which is presently an expanding industry (Augostine & Avery 2022). Compared to antifungal drugs made from synthetic chemicals, those made from natural ingredients have many advantages and lower toxicity levels.

In this study, the minimum inhibitory concentration was determined using the liquid microdilution method. According to a study conducted by Balouiri et al. (2016), this particular method is widely regarded as the most appropriate approach due to its ability to estimate the necessary concentration of antimicrobial agents. The findings of this study showed that *Selaginella plana* extract at specific concentrations (100%, 50%, and 25%) can inhibit the growth of *Candida albicans*. Consequently, it can be classified as a fungistatic agent. However, *Selaginella plana* extract at low concentrations cannot inhibit *Candida albicans*. The fungistatic ability of *Selaginella plana* is attributed to the presence of an active compound that inhibits the growth of *Candida albicans*, with the inhibitory effect becoming more evident as the concentration of the plant extract increases. All active compounds in Selaginella show high potential as effective antimicrobials against *Candida albicans* (Cao et al. 2015).

In a previous study, Janeczko et al. (2022) examined the antifungal activity of quercetin, a flavonoid compound, against *Candida albicans*. The study revealed a range of minimum inhibitory concentrations between 2 mg/mL and 256 mg/mL. The activity of quercetin causes inhibition of hyphae formation, enhances membrane permeability, and induces cell leakage in *Candida albicans*. The plasma membrane serves as a protective barrier that plays a crucial role in preserving microbial cell viability. However, the active compound can disrupt adenosine triphosphate (ATP) synthesis, leading to obstruction of extracellular transportation across the membrane. In addition, it is widely known that saponin compounds can destabilize the integrity of cellular membranes, thereby inducing osmotic stress that leads to the release of cell organelles and subsequent cell lysis (Nuraeni et al. 2020).

In the minimum fungicidal concentration test conducted in this study, *Selaginella plana* extract showed an effect against *Candida albicans* at 100% concentration. This result was proven by the lack of colonies that proliferated on the agar media. This might be because *Selaginella plana* contains secondary metabolites such as flavonoids, tannins, and saponins (Miftahudin et al. 2015). Other studies have focused on the effects of flavonoid compounds on *Candida albicans*. These studies found that amentoflavone increased reactive oxygen species (ROS) while also impeding the ergosterol biosynthesis of *Candida* fungi (Nascimento et al. 2018). Consequently, this disruption leads to mitochondrial dysfunction and cell death. Additionally, flavonoids (e.g., isoquercitrin and flavonol) can cause changes in cell membrane permeability, thus inhibiting cell growth (Nguyen et al. 2021).

In a previous study conducted by Shariati et al. (2022), it was shown that terpenoids have the capability to destroy the cell membrane structure of *Candida albicans*. These compounds can block the respiratory chain by inhibiting succinate dehydrogenase, an enzyme attached to the inner mitochondrial membrane of *Candida albicans*. Furthermore, it was determined that the *Selaginella plana* extract at concentrations ranging from 50% to 3.125% has no fungicidal effects against *Candida albicans*. Resistance to antifungal drugs can be attributed to various factors, including point mutations, increased expression levels of the lanosterol 14-α-demethylase (ERG11) gene, and enzymatic changes within the ergosterol biosynthetic pathway (Alizadeh et al. 2017). The 14α-demethylase (Erg11p) gene is involved in the biosynthesis of ergosterol, which serves as the primary sterol in the fungal cell membrane. In addition, the ability of *Candida albicans* to form biofilms is an essential factor in the development of resistance to antifungal drugs (Alikhani et al. 2022).

Strength and limitations

The strength of this study is the novel application of *Selaginella plana* extract as an antifungal agent specifically targeting *Candida albicans*, which has not been reported in previous research. Therefore, this study represents the first report of the inhibitory and fungicidal properties of *Selaginella plana* extract against the growth of *Candida albicans*, as demonstrated by the minimum inhibitory concentration and minimum fungicidal concentration test results. In addition, to determine the antifungal efficacy of *Selaginella plana*, the researchers used the agar dilution method to observe differences in the inhibitory and fungicidal power of the extract at different concentrations. The limitation of this study is that the researchers have yet to explicitly examine the ability of *Selaginella plana* bioactive compounds by observing the absorbance using spectrophotometry. Therefore, the measurement of absorbance is necessary to accurately determine the turbidity of the fungal suspension.

CONCLUSION

Selaginella plana extract possesses inhibitory properties against the growth of *Candida albicans*. At the same time, its fungicidal ability is effective only in the form of undiluted, pure extracts. Further research is necessary to explore the underlying mechanism of the active compounds present in *Selaginella plana* extract with regards to their efficacy as antifungal agents.

Acknowledgment

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Conflict of interest

None.

Ethical consideration

The ethical approval for this research was issued by the Health Research Ethics Committee, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia, with certificate No. 766/HRECC. FODM/XII/2019 on 17/12/2019.

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None.

Author contribution

All authors contributed to the conception and design of the study, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, provision of the study materials, and collection and assembly of the data.

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Systematic Review

EFFICACY, SAFETY, AND CLINICAL OUTCOMES OF SPLENORENAL SHUNT SURGERY AS A THERAPEUTIC INTERVENTION FOR PORTAL HYPERTENSION PATIENTS

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ABSTRACT

Portal hypertension is the second most common gastrointestinal bleeding in cirrhosis and non-cirrhosis patients. The splenorenal shunt surgery is a potential intervention that may be considered for portal hypertension patients with clinical symptoms such as upper gastrointestinal bleeding caused by the rupture of gastro-esophageal varices. The objective of the procedure is to prevent long-term complications of portal hypertension, such as biliopathy. In this study, the researchers aimed to analyze the efficacy, clinical outcomes, and safety of splenorenal shunt surgery in portal hypertension patients. This study used a qualitative methodology with a systematic review framework. The sources were obtained from electronic search databases, i.e., PubMed, Google Scholar, and ScienceDirect, using "Efficacy," "Safety," and "Clinical Outcomes" as keywords that are related to splenorenal shunt surgery in portal hypertension patients. The researchers set specific criteria for the inclusion and exclusion of articles. The inclusion criteria were articles that met the following requirements: (1) retrospective case studies, retrospective cohort studies, or prospective cohort studies published from 2018 to 2023; (2) the topic of splenorenal shunt surgery in hepatic portal vein hypertension, with a specific evaluation of their efficacy, safety, and clinical outcomes that were established through clinical data validation. Ten studies were selected according to the inclusion criteria. This systematic review revealed the efficacy of the splenorenal shunt procedure with favorable outcomes. The success rate of splenorenal shunt surgery in reducing the clinical symptoms of portal hypertension varied between 66% and 100%. The prevailing complications observed in this study were shunt thrombosis, rebleeding, and thrombocytopenia. However, notable improvements could be achieved with general treatment. In terms of short- and long-term clinical outcomes, the splenorenal shunt procedure demonstrated favorable results. It can be concluded that splenorenal shunt surgery provides excellent clinical outcomes and should be considered a viable treatment option for patients with both cirrhotic and non-cirrhotic portal hypertension.

Keywords: Portal hypertension; splenorenal shunt surgery; efficacy; clinical outcomes; human and health

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Highlights:
1. This study systematically reviewed the efficacy, clinical outcomes, and safety of the splenorenal shunt procedure with a comprehensive and meticulous approach.
2. The splenorenal shunt procedure is an innovative surgical intervention that offers a viable option for the management of portal hypertension.
3. The findings of this study may improve clinical decision-making by helping healthcare professionals more effectively evaluate portal hypertension management strategies.

INTRODUCTION

Hepatic portal vein hypertension is a medical disorder that is characterized by an increase in pressure inside the hepatic portal venous system. The examination of this condition can be conducted through the measurement of the elevated portal pressure gradient, which represents the difference between the pressures observed in the portal vein and those in the inferior vena cava or hepatic vein (Gunarathne et al. 2020, Oliver et al. 2023). Normally, the difference in portal pressure gradient is ≤5 mmHg. If the pressure gradient is ≥6 mmHg, it can be inferred that portal hypertension is present. Additionally, if the pressure is greater than 10 mmHg, clinical symptoms of portal hypertension typically appear. Meanwhile, if the pressure is 5–9 mmHg, it is usually indicative of a subclinical condition. Although the exact cause of this condition remains uncertain, it is classified into prehepatic, intrahepatic, or post hepatic causes (Gunarathne et al. 2020, Gioia et al. 2020). From an epidemiological standpoint, the most common cause is cirrhosis of the liver. However, schistosomiasis is the predominant factor in portal hypertension cases in Africa.

Typical features of portal hypertension are varices or variceal bleeding. The veins in the oesophagus normally carry blood into the hepatic portal vein. However, in cases of hypertension, these veins enlarge and carry blood away from the portal vein, which is commonly referred to as an oesophageal varices (Rockey 2017). These blood vessels may burst, resulting in the occurrence of hematemesis. In addition, the features of cirrhotic patients with portal hypertension can trigger ascites. Varicose or ascitic bleeding in cirrhotic patients is called decompensation. The most effective investigation method, albeit invasive, is the measurement of the hepatic vein pressure gradient. Clinically, if left untreated, portal hypertension can result in ascites, bleeding from varicose veins, and hepatic encephalopathy because the liver cannot remove ammonia due to collateral circulation, which causes the patient to lose consciousness. The treatment of portal hypertension depends on its underlying cause (Kibrit et al. 2018). Various therapies may be used, such as anticoagulants in confirmed thrombosis cases, non-selective β-blockers, endoscopic ligation of varices in cirrhotic patients with large varicose veins or a risk of stigmata, implantation of a transjugular intrahepatic portosystemic shunt (TIPS) for acute variceal bleeding, antibiotics for peritonitis prevention, and other symptomatic treatments. The novel surgical-based therapy that has currently been developed is splenorenal shunt surgery (Gao et al. 2020, Iwakiri & Trebicka 2021).

A splenorenal shunt surgery is a surgical procedure that attaches the splenic vein to the left renal vein. This surgical procedure is typically performed to treat hepatic portal hypertension and its common complications. The procedure consisted of two types, i.e., proximal splenorenal shunt (PSRS) and distal splenorenal shunt (DSRS) (Papandria et al. 2019, Yao et al. 2021). Portal hypertension patients with clinical symptoms, such as upper gastrointestinal bleeding due to rupture of esophago-gastric varices, may consider the option of undergoing splenorenal shunt surgery. This surgery can prevent severe complications, such as biliopathy. In addition, this surgical procedure can also restore hepatic portal vein pressure to its normal levels and reduce postoperative morbidity and mortality (Dasanayake et al. 2020). Previous studies have demonstrated excellent outcomes, safety, and effectiveness of PSRS and DSRS, albeit with some reported side effects of little significance. However, it is imperative to conduct a systematic review to provide a comprehensive overview regarding the safety, efficacy, and clinical outcomes of splenorenal shunt surgery. Therefore, this research aimed to present a comprehensive review that investigated the safety, clinical outcomes, and efficacy of splenorenal shunt surgery in portal hypertension patients.

MATERIALS AND METHODS

As recommended by Moher et al. (2015), this study was conducted with a review protocol that adhered to the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines. The reviewers of this research undertook the study selection and data extraction independently. All data were recorded electronically and organized in tabular format. The researchers assessed the feasibility of the literature. All of the researchers examined the titles, abstracts, and entire contents of the acquired articles.

The initial search of literature was conducted using a keyword extraction algorithm. The search started by querying indexed databases, including Google Scholar, ScienceDirect, and PubMed, using the specified keywords "Efficacy," "Safety," and "Clinical Outcomes" in relation to splenorenal shunt surgery in portal hypertension patients. The search was limited to publications within the last five years without any data discrimination. The researchers weed out duplicate articles after compiling results from the initial search. After getting rid of articles that were not related to the keywords, the researchers thoroughly selected articles that had the potential to be a source of data according to the inclusion and exclusion criteria. In addition to the PRISMA guidelines, this study adhered to the Cochrane Handbook for Systematic Reviews of Interventions (Page et al. 2021). The articles that

met the inclusion criteria comprised retrospective case studies, retrospective cohort studies, and prospective cohort studies that were published between 2018 and 2023. The primary focus of the studies must be centered around splenorenal shunt surgery in hepatic portal vein hypertension, with a detailed analysis of its efficacy, safety, and clinical outcomes, which were established through clinical data validation. The exclusion criteria consisted of the following: article duplicates; articles that were not compatible with the specified keywords; articles that did not provide information on efficacy, safety, and clinical outcomes of splenorenal shunt surgery; letters, review articles, or commentaries; and articles in languages other than English.

The data were extracted from the selected journal articles according to the aforementioned inclusion criteria. The researchers synthesized and arranged the data in tabular format by categorizing them by numerous variables, including study design, year of publication, country of origin, and clinical characteristics (i.e., age, sex, etiology of portal hypertension, complications, and medical indications for splenorenal shunt procedure). When a particular data point was absent from the selected literature, it was denoted as "NA" (an abbreviation for "not available") within the tables (Espinós et al. 2018).

RESULTS

The researchers discovered a total of 150 publications that were published throughout the period of 2018–2023. These articles were retrieved from three different search engines, i.e., PubMed (n=100), ScienceDirect (n=25), and Google Scholar (n=25). A total of 140 records were excluded from the study due to various reasons, including duplicate articles (n=50), non-English articles, failure to be consistent with the abstract (n=10), the absence of full text (n=50), and articles that did not address the specific subject matter as defined by the researchers (n=30). Thus, a total of ten articles were deemed relevant to our topic and met the inclusion criteria. The flowchart of the article selection process can be seen in Figure 1.

The characteristics of the publications that were selected are presented in Table 1. Of the 150 articles, there were ten studies that reported the safety, efficacy, and clinical outcomes of splenorenal shunt surgery. In the ten selected articles that were identified and screened, it was found that the majority of the studies came from the United States. The remaining studies were from Germany, India, South Africa, Pakistan, and France. The studies reported splenorenal shunt surgery in a total of 238 portal hypertension patients. All of the selected studies were retrospective cohort studies. The data exhibited a prevalent representation of male patients. The age of onset upon diagnosis was 5.5–27 years. The causes of hepatic portal vein hypertension in the studies included schistosomiasis, cystic fibrosis, portal vein thrombosis, and splenic vein ligation during pancreaticoduodenectomy. The most common complications related to portal hypertension were varices bleeding, hypersplenism, pancytopenia resulting from hypersplenism, and advanced decompensated liver cirrhosis. The literature revealed that the indications most frequently reported for splenorenal shunt surgery included unsuccessful transjugular intrahepatic porto-systemic shunt insertion, hepatic portal venous thrombosis, cystic fibrosis, decompensated liver cirrhosis, recurrent gastrointestinal bleeding, and hypersplenism.

The efficacy and safety of splenorenal shunt surgery were reported in the ten selected articles included in this systematic review. Several studies presented both the efficacy and safety of splenorenal shunt surgery, whereas other studies solely focused on either efficacy or safety. Table 2 summarizes the findings regarding the safety and efficacy of splenorenal shunt surgery as a surgical intervention for portal hypertension.

The findings of this systematic review indicated that splenorenal shunt surgery exhibited efficacy, as evidenced by the favorable results seen in the selected studies. The success rates of the splenorenal shunt procedure in reducing clinical symptoms of portal hypertension ranged from 66% to 100%. The studies conducted by Nordmann et al. (2021) and van Praet et al. (2021) yielded success rates of 100%. The later study further revealed a complete shunt patency rate of 100%, with no instances of shunt thrombosis, re-intervention for variceal bleeding, or mortality. According to the study by Gupta et al. (2022), a success rate of 88% was seen over the follow-up period, with no reported cases of thrombosis development. Comparable results were also documented in another study that was reviewed in this research. The study reported that the primary patency rates of splenorenal shunts at one-year and five-year intervals were observed to be 71% and 66%, respectively (Cortez et al. 2019).

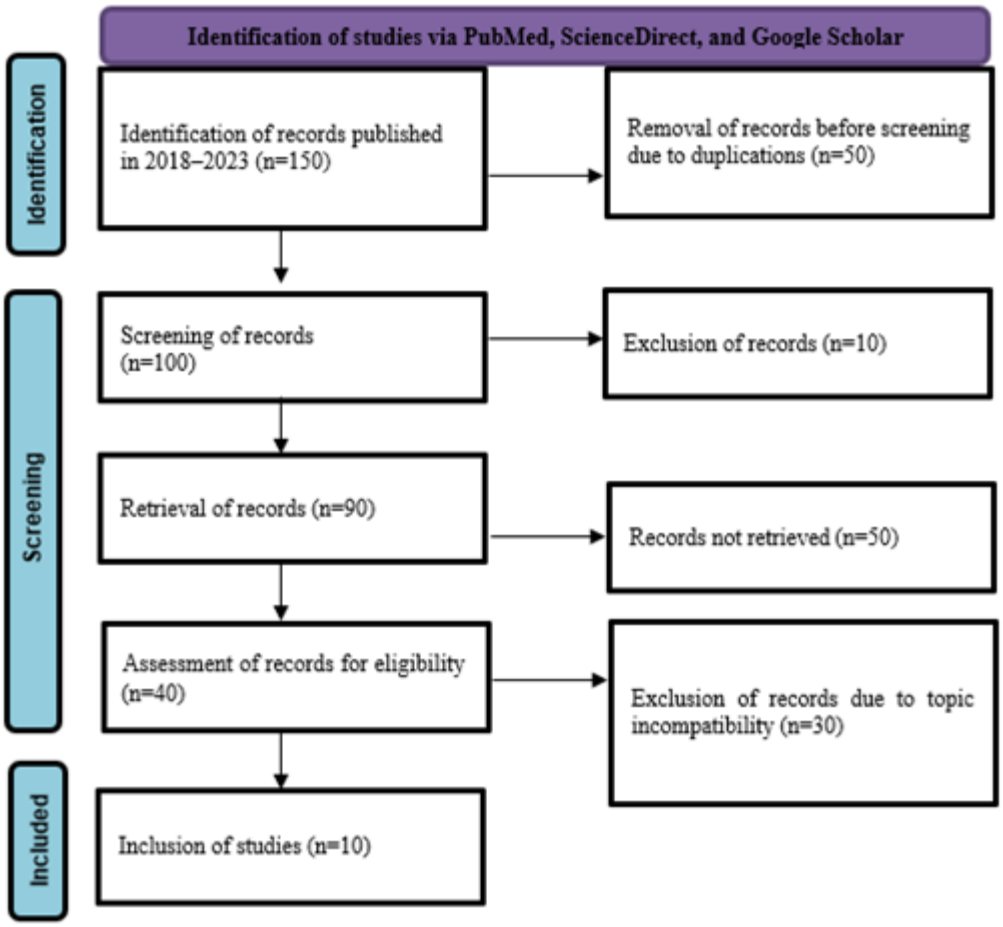

Figure 1. PRISMA flowchart of the article selection process.

The ten selected studies provided substantial evidence on the safety of splenorenal shunts in the treatment of portal hypertension. Several studies mentioned a number of complications that arose during the surgical procedures, including transient thrombocytosis, shunt and portal vein thrombosis, rebleeding, and thrombocytopenia. Some of the studies also reported increased risks of mortality due to complications such as arterial rupture, pulmonary embolism, and multiorgan failure after hemorrhagic shock. However, it is important to consider that the splenorenal shunt procedure in patients with portal hypertension demonstrated no long-term complications such as thrombosis or stenosis. Additionally, this procedure showed improvements in various aspects, including variceal bleeding, hypersplenism, hemoglobin and leukocyte levels, ascites, spontaneous bacterial peritonitis, jaundice, hepatorenal syndrome, hepatopulmonary syndrome, and portoencephalopathy. The improvements were also observed in the laboratory test results of liver functions, including total bilirubin, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), and alkaline phosphatase. The occurrence of postoperative rebleeding was observed, albeit with limited reported cases in the selected studies.

Table 3 presents a comprehensive overview of the outcomes of splenorenal shunt surgery in the management of portal hypertension, as reported in the selected studies. The findings of this systematic review indicated that several studies demonstrated favorable clinical outcomes. The clinical outcomes of splenorenal shunt surgery were categorized into three distinct phases: preoperative, perioperative, and postoperative. The majority of the studies revealed similar preoperative outcomes, which included various conditions such as hypersplenism, Child-Pugh scores of 5 (class A), MELD scores of 3-28, pancytopenia, esophageal varices of grade II-IV according to Paquet's classification, advanced decompensated liver cirrhosis, ascites, spontaneous bacterial peritonitis, portal hypertensive biliopathy, hepatorenal syndrome, hepatopulmonary syndrome, and portosystemic encephalopathy. The procedures reported by the studies were proximal and distal splenorenal shunts.

Table 1. Characteristics of the selected studies.

Authors (Year)	Country	Study Design	n	Gender (Male)	Age (median / mean)	Etiologies	Complications	Indications
Nordmann et al. (2021)	United States	Retrospective case study	2	1	27	Hepatosplenic schistosomiasis	Variceal bleeding	Unsuccessful TIPS insertion, hepatic portal venous thrombosis
Lemoine et al. (2019)	United States	Retrospective cohort	5	3	6.9	Cystic fibrosis	Hypersplenism, variceal bleeding, thrombocytopenia	Portal hypertension related to cystic fibrosis
			9	8	10.7 ±4.2	Cystic fibrosis and liver disease	Advanced decompensated liver cirrhosis, hypersplenism, variceal bleeding	Decompensated liver cirrhosis
van Praet et al. (2021)	Germany	Retrospective Cohort	14	7	15	Portal vein thrombosis	Variceal bleeding	Portal vein thrombosis
Anand et al. (2020)	India	Prospective Cohort	25	19	18.6	Extrahepatic portal vein obstruction, non-cirrhotic portal fibrosis	Variceal bleeding	Recurrent GI bleeding and hypersplenism
Khamag et al. (2023)	South Africa	Retrospective Cohort	23	5	12	Extrahepatic portal vein obstruction	Hypersplenism, variceal bleeding	Recurrent GI bleeding and hypersplenism
Gupta et al. (2022)	India	Retrospective cohort	47	23	22	Extrahepatic portal vein obstruction, non-cirrhotic portal fibrosis	Variceal bleeding	Recurrent GI bleeding and hypersplenism
Rehman & Nazir (2019)	Pakistan	Retrospective Cohort	9	11	6	Extrahepatic portal hypertension	Variceal bleeding	Recurrent GI bleeding and hypersplenism
Addeo et al. (2020)	France	Retrospective Cohort	78	NA	63±8.1	Splenic vein ligation during pancreaticoduodenectomy	NA	Carcinoma of pancreas
Cortez et al. (2019)	United States	Retrospective Cohort	10	5	8.1	Extrahepatic portal vein thrombosis, chronic liver disease	NA	Recurrent GI bleeding
Woerner et al. (2018)	United States	Retrospective Cohort	16	NA	5.5	NA	NA	NA

Short-term complications during surgery were rarely reported. However, the study by Cortez et al. (2019) found perioperative outcomes with a median operating time of 390.5 minutes, estimated blood loss of 125 mL, portocaval gradient, pre-shunt mesenteric pressure of 28.7 mmHg, post-shunt mesenteric pressure of 22.8 mmHg, and a mesenteric pressure of 5.1 mmHg. The postoperative outcomes showed a hepato-encephalopathy grade of 0, a constant Child-Pugh score of 5 (class A), and improvements in esophageal varicose and thrombocytopenia. The life expectancy rate was found to be 80% with improved liver functions, as shown by the levels of bilirubin, SGOT, SGPT, and alkaline phosphatase. There was no occurrence of variceal bleeding after shunt, decreased spleen size, ascites, spontaneous bacterial peritonitis, jaundice, or hepatorenal syndrome.

The duration of follow-up ranged from 14 to 18.2 months. During the follow-up, variceal regression (100%), total regression (40%), and thrombosis (20%) were found in the patients who underwent splenorenal shunt surgery. The classification of clinical outcomes following shunt surgery in the study conducted by Cortez et al. (2019) included two categories, i.e., short-term outcomes and long-term outcomes. The short-term outcomes were the length of stay (9.5 days), postoperative transfusion (30%), wound complications (0%), early shunt thrombosis (20%), and unplanned reoperation (10%). Meanwhile, the long-term complications were recurrent bleeding within 60 days postoperatively (10%), readmission (30%), mortality (0%), appropriate growth development in terms of length (70%) and weight (90%), shunt complications (20%), and postoperative liver transplantation (30%).

Table 2. Efficacy and safety of splenorenal shunt surgery for portal hypertension.

Authors (Year)	Country	Efficacy	Safety
Nordmann et al. (2021)	United States	Success rate: 100%	Post-surgery complications: • Transient thrombocytosis • Shunt and portal vein thrombosis
Lemoine et al. (2019)	United States	Effective improvement of pulmonary hypertension and hypersplenism in CFLD patients	• No long-term shunt complication; no shunt stenosis or thrombosis • No clinical finding of hepatic encephalopathy, although levels of ammonia were not consistently measured
van Praet et al. (2021)	Germany	• Median duration of follow-up: 3 years • Survival rate in the last follow-up: 100% • Shunt thrombosis: 0% • Maximum flow velocity: 46 cm/s • Shunt patency rate: 100% • Re-intervention due to variceal bleeding: 0%	• No perioperative complication • Improvement of esophageal variceal bleeding, hypersplenism, and the counts of hemoglobin, thrombocytes, and leukocytes • No ascites, spontaneous bacterial peritonitis, jaundice, hepatorenal syndrome, or portoencephalopathy • Improvement of liver functions, as shown by the examination of bilirubin, SGOT, SGPT, and alkaline phosphatase
Anand et al. (2020)	India	Success rate: 80%	• Rebleeding rate 48 hours post-surgery: 8% • Thrombosis of the splenic vein and superior mesenteric: 4%
Khamag et al. (2023)	South Africa	Success rate: 73%	• No complication post-surgery • Complications found during follow-up: GI bleeding and thrombosis
Gupta et al. (2022)	India	Success rate: 88% No development of thrombosis after follow-up	• Shunt thrombosis • Thrombocytopenia
Rehman & Nazir (2019)	Pakistan	Success rate: 88.8%	Rebleeding
Addeo et al. (2020)	France	Success rate: 69.2%	Mortality due to grade C pancreatic fistula with arterial rupture, pulmonary embolism, and multiorgan failure after hemorrhagic shock due to arterial bleeding
Cortez et al. (2019)	United States	One-year primary patency rate: 71% Five-year primary patency rate: 66%	Complications: thrombosis and shunt stenosis
Woerner et al. (2018)	United States	Success rate: 95.2%	Complication: shunt stenosis

Table 3. Outcomes of splenorenal shunt surgery for portal hypertension

Author	Country	Outcomes		
		Preoperative	Perioperative	Postoperative
Nordmann et al. (2021)	United States	• Follow-up period: 17–23 months • Spleen length: 211–213 mm • Child-Pugh score: 5 (A) • PELD: 9-12 • Initial platelet counts: 43-53 n/L • Esophageal varicose and rectal varicose with red spots, and • Paquet's classification grade: II-III	Proximal splenorenal shunt with splenectomy	• Hepatic Encephalopathy grade: none • Child-Pugh score: 5 (A) • Gastroesophageal varicose: none • Rectal varicosis regradient • Paquet's classification grade: I • Reduction of Paquet's classification grade: 2 • Latest platelet count: 200-518 n/L
Lemoine et al. (2019)	United States	Cystic fibrosis patients: • Hypersplenism • Thrombocytopenia • Well-preserved liver function (albumin, INR, and total bilirubin) • MELD/PELD score: 3±6 • Esophageal varicose • Paquet's classification grade: III-IV CFLD patients: • Advanced decompensated liver cirrhosis • Hypersplenism • Variceal bleeding • PELD/MELD score: 28±4	Distal splenorenal shunt without any immediate or early perioperative surgical complications	• Survival rate: 80% • One mortality case due to pulmonary complication of cystic fibrosis • No postoperative rebleeding • Improvement of splenism • No difference between preoperative and postoperative platelet counts, with spontaneous improvement at the last follow-up • Compression of left renal vein, impaired drainage to the IVC, and pressure gradient of 5-6 mmHg that can not be improved with angioplasty
van Praet et al. (2021)	Germany	• Esophageal variceal bleeding • Hypersplenism • Anemia, thrombocytopenia, and leukopenia • Ascites • Spontaneous bacterial peritonitis • Portal hypertensive biliopathy (abnormal liver function test result) • Hepatorenal syndrome • Portosystemic encephalopathy	Distal splenorenal shunt without reported immediate or short-term perioperative surgical complications	• Improvement of esophageal variceal bleeding, hypersplenism, and the counts of hemoglobin, thrombocytes, and leukocytes • No ascites, spontaneous bacterial peritonitis, jaundice, hepatorenal syndrome, or portoencephalopathy • Improvement of liver function laboratory test results (alkaline phosphatase, SGPT, SGOT, and bilirubin)
Anand et al. (2020)	India	Esophageal variceal bleeding	Proximal splenorenal shunt without immediate or short-term perioperative surgical complications	Follow-up (n=20) • Variceal regression: 100% • Complete variceal regression: 40% • Shunt thrombosis: 20% • Rebleeding: 10% • Hepatic encephalopathy: 0%
Khamag et al. (2023)	South Africa		Distal splenorenal shunt without immediate or short-term perioperative surgical complications	Follow-up (n=21): • Improvement of platelet counts and liver functions • Median period of follow-up: 129 months
Gupta et al. (2022)	India	• Esophageal variceal bleeding • Hypersplenism • Pancytopenia	Proximal splenorenal shunt, with intraoperative blood loss of 500 cc	• Follow-up period: 3 years • Shunt thrombosis: 37.2% • Complete variceal regression: 59.7%

Rehman & Nazir (2019)	Pakistan	• Bleeding episodes: 100% • Platelet counts: 57.4±22.2 • Spleen size: 15.6±2.4	Distal splenorenal shunt without operative complications, such as bleeding, injury to the surrounding tissue, wound infection, or encephalopathy	• Bleeding episodes: 11.1% • Platelet counts: 94.4±34.4 • Spleen size: 15.1±2.5
Addeo et al. (2020)	France		Left splenorenal shunt with a perioperative transfusion rate of 66%	• Survival rate: 18.2 months • Mortality rate: 1.28% • Morbidity rate: 30%
Cortez et al. (2019)	United States	• Esophageal varices • Gastrointestinal bleeding • Refractory ascites • hepatopulmonary syndrome • Hepatorenal syndrome • Hepatic encephalopathy	Distal splenorenal shunt, with the following perioperative outcomes: • Median operative time: 390.5 minutes • Estimated blood loss: 125 mL • Portocaval gradient: mesenteric pressures of 28.7 mmHg (pre-operative), 22.8 mmHg (post-operative), and 5.1 mmHg	Median period of follow-up: 14 months Short-term outcomes: • Length of stay: 9.5 days • Red blood cell transfusion: 30% • Wound complication: 0 • Early shunt thrombosis: 20% • Unplanned reoperation: 10% • Other complications: 20% Long-term outcomes: • Recurrent GI bleeding within 60 days post-surgery: 10% • Readmission within 60 days post-surgery: 30% • Mortality within 60 days post-surgery: 0% • Appropriate growth development (length, weight): 70%, 90% • Shunt complication: 20% • Liver transplantation: 30%
Woerner et al. (2018)	United States	NA	NA	• Stenosis: 83.33% • Platelet count of <50,000/mm3: 12.5% • Platelet count of <100,000/mm3: 83.33% • Spleen diameter increase of >10%: 91.66% • Recurrent variceal bleeding: 20.83%

Notes: NA=Not available; PELD=Pediatric end stage liver disease model; MELD=Model for end stage liver disease; INR=International normalized ratio; DSRS=Distal splenorenal shunt; IVC=Inferior vena cava; SGOT=Serum glutamic oxaloacetic transaminase; SGPT=Serum glutamic pyruvic transaminase; GI=Gastrointestinal.

DISCUSSION

This systematic review examined the data of 238 patients obtained from the selected articles published in 2018–2023. The collected data represented the clinical outcomes, efficacy, and safety of splenorenal shunt surgery as the treatment of portal hypertension. As previously stated, the majority of the studies consistently indicated that bleeding and rebleeding were the predominant severe complications associated with hepatic portal vein hypertension resulting from either cirrhosis or non-cirrhosis. These complications had a significant impact on the prognosis and clinical outcomes of splenorenal shunt surgery. A broad range of therapeutic options were widely used, spanning from pharmacological to non-pharmacological interventions (Gairing et al. 2021, Yao et al. 2021). The pharmacological intervention exhibits favourable effectiveness in the short-term management of portal hypertension. However, this method does not change the overall portal vein blood flow or pressure, hence leading to the potential recurrence of varicose veins. There are ongoing developments in the field of surgical interventions, including the development of various procedures such as splenorenal shunt surgery (both proximal and distal) and transjugular intrahepatic portosystemic shunts. These interventions have been reported to have the potential to reduce mortality and morbidity rates in the specific case being discussed (Terwagne et al. 2022).

The findings of this systematic review indicated that the entire literature confirmed the excellent efficacy, safety, and clinical outcomes of splenorenal shunt surgery in patients with portal hypertension. The aforementioned results are consistent with the meta-analysis conducted by Yao et al. (2021), who revealed that transjugular intrahepatic portosystemic shunts and distal splenorenal shunts exhibited a greater capacity to decrease rebleeding rates when compared to other therapeutic approaches. Several other studies have indicated that the use of mixed treatments may

yield more favourable outcomes. The suggested surgical interventions for hepatic portal vein hypertension in the presence of portal vein thrombosis are laparoscopic splenectomy and proximal splenorenal shunts (Gómez et al. 2021). A combination of a modified surgical technique with proximal splenorenal shunts has also been discovered as an advantageous therapeutic approach for managing portal vein thrombosis in patients with non-cirrhotic portal hypertension. This treatment strategy has demonstrated efficacy in preventing rebleeding, as supported by studies conducted by Gupta et al. (2019), Irawan & Mulyawan (2019), and Terwagne et al. (2022).

According to a study conducted by Malviya et al. (2022), splenorenal shunt surgery falls within the category of unconventional shunts, which can be used effectively and safely with favourable outcomes. This argument is supported by the results of various additional investigations. In the study by Biju et al. (2019), splenorenal shunt surgery was proven to be effective in lowering the risks of mortality and morbidity associated with portal hypertension. Moreover, the presence of abnormalities in the renal veins did not prevent the implementation of proximal splenorenal shunt surgery, even with different modifications. End-to-end interposition of proximal splenorenal shunt grafts is the ideal option, as it provides advantages in terms of shunt patency and clinical outcomes. In contrast, the study conducted by Yi et al. (2021) found that spontaneous splenorenal shunts as a surgical intervention for managing hepatic portal vein hypertension in cirrhosis had a detrimental effect on hepatic function and resulted in an increased mortality rate. Nevertheless, splenorenal shunt surgical intervention remains significant in the prevention of spontaneous splenorenal shunts in patients with cirrhosis. According to the results of the study conducted by Ravindranath et al. (2020), the intervention technique implemented in patients with comorbid cholangiopathy was effective in managing portal hypertension. However, it should be noted that this intervention did not lead to improvements in cholangiopathy or peribiliary collateral.

Strength and limitations

One notable aspect of this study is its pioneering nature, as it is the first-ever comprehensive analysis of splenorenal shunt surgery in the management of portal hypertension as far as the author is concerned. However, the scarcity of literature limited the information available regarding the splenorenal shunt procedure as a surgical intervention for portal hypertension.

CONCLUSION

This systematic review provides evidence supporting the efficacy, safety, and clinical results of splenorenal shunt surgery for treating portal hypertension. Patients diagnosed with either cirrhotic or non-cirrhotic portal hypertension may consider this surgical strategy to manage their condition.

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Conflict of interest

None.

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Author contribution

YES contributed to the conception, design, and final approval of the article. HS contributed to the analysis and interpretation of the data. DJSW contributed to the drafting of the article. IP and JJD contributed to the critical revision of the article for important intellectual content. SPWBN and IGAMAP contributed to the provision of administrative, technical, and logistic support. KPY contributed to the collection and assembly of data.

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Systematic Review

THE EFFECT OF CHILDHOOD OBESITY ON PSYCHOMOTOR BEHAVIOR

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ABSTRACT

Childhood obesity is a complex problem, and its prevalence among school-age children has been found to have a greater impact on their physical well-being compared to their learning difficulties. In some cases, teachers or schools may not pay enough attention to issues that have the potential to affect or delay the psychomotor development of children. Therefore, this study aimed to provide enhanced comprehension regarding the effect of childhood obesity on psychomotor behavior. This study used a systematic review methodology to synthesize information regarding the effect of obesity on children's health and development. The information and data presented in this study were obtained from several scientific sources accessed through online libraries such as PubMed, ScienceDirect, and the Cochrane Library. A comprehensive search was conducted to identify scholarly publications, resulting in a total of 815 papers published between 2013 and 2022. The selected papers exclusively consisted of original research articles that primarily focused on investigating the relationship between obesity in children aged 5–14 years, their psychomotor abilities, and development outcomes. Following the screening process, five studies were found to meet the specified criteria. The findings of the selected studies revealed a substantial resemblance, specifically the correlation between childhood obesity and poor motor skills. Furthermore, the selected studies discovered that an increase in body fat was commonly associated with a rise in total body weight, or the relative body mass index (BMI), in both adults and children. Some studies demonstrated significant differences in the performance of diverse psychomotor variables according to children's BMI. According to the results of the studies, children with a higher BMI showed inferior performance in motor activities as a result of compromised body control and balance that inhibited the children's movement. This systematic review concludes that obesity affects the psychomotor behavior of children.

Keywords: Obesity; psychomotor; movements; children

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Highlights:
1. This study demonstrated an association between childhood obesity and poor psychomotor function, a concern that necessitates a greater effort from multiple stakeholders to improve child health.
2. Given the rising frequency of childhood obesity, this study provides an enhanced understanding of the impact of overweight and obesity on psychomotor abilities.

INTRODUCTION

The classification of overweight and obesity in children and adolescents is determined by employing age- and sex-specific nomograms to calculate the body mass index (BMI). Children with a gender-specific BMI above the 95th percentile are considered obese. Meanwhile, children with a BMI of the 85th percentile or above but below the 95th percentile are classified as overweight. This categorization is contingent on the risks of comorbidities that are commonly associated with obesity (Ghosh 2014). The prevalence of obesity is rising globally, resulting in detrimental effects on children's health, motor growth, and participation in physical activities. Childhood obesity is a global

phenomenon that affects all socioeconomic groups, regardless of age, gender, or ethnicity. The etiology of obesity in children is multifactorial and includes genetic, neuroendocrine, metabolic, psychological, environmental, and sociocultural factors (Berleze & Valentini 2022). The growth of children may subsequently result in a growing waistline. However, a lack of movement or activity leaves them more susceptible to the development of obesity and related health issues. Obesity is considered an independent risk factor for various diseases and mortality (Chahar 2014). It poses a serious public health concern and threatens the feasibility of essential medical care in many countries. Childhood obesity has been found to cause comorbidities, such as diabetes and cardiovascular disorders (Sahoo et al. 2015). According to a study by García et al. (2013), there has been a global rise in the prevalence of overweight and obesity among children. The prevalence rate increased from 4.2% in 1990 to 6.7% in 2010. The numbers of overweight and obese children aged 5 to 12 years in Indonesia were found to be even higher. The findings of the 2018 Basic Health Research revealed that the percentages of childhood overweight and obesity in Indonesia were 10.4% and 10.7%, respectively (Badan Penelitian dan Pengembangan Kesehatan 2020).

Children who are classified as obese may encounter difficulties to engage in sports and other recreational activities alongside their non-obese peers. This is because of their comparatively lower levels of real and perceived motor skill competence. The development of children's motor skills is contingent upon the complexity of the activities as well as the opportunities and resources that are accessible within the surrounding environment (Kakebeeke et al. 2017, Awad & Aneis 2022). Obesity is a physical problem that has the potential to decline or slowdown social, emotional, linguistic, and cognitive developments. The processes of intellectual and emotional development are simultaneously interrelated. The presence of an inhibitor in a specific development can potentially have a detrimental effect on other developments. The incidence of obesity in children is influenced by various factors, including lifestyle changes, dietary habits, and parental behaviors, which have been identified as contributors to the increasing prevalence of obesity (Kanazawa 2013, Agudo & Marcenaro-Gutierrez 2021).

One plausible hypothesis argues that obesity may have an impact on the physical development of children, potentially influencing their psychomotor skills and cognitive capacities. Obesity has been found to disrupt coordinated movements in children, particularly in regards to motor development and musculoskeletal issues (Srinag et al. 2020, Zacks et al. 2021). Children who are classified as obese or overweight have inadequate gross and fine motor control, as well as delayed motor growth. Furthermore, obese children commonly experience neuromusculoskeletal deficits, which might impact their walking movements and hinder their interest or ability to engage in physical activities and exercise. Obesity has been linked to a higher risk of musculoskeletal disorders, which leads to a higher risk of musculoskeletal problems. This condition could potentially be attributed to the increased strain on joints and bones caused by excessive body weight (Wang et al. 2016, O'Malley et al. 2021). In light of the aforementioned risks associated with obesity, this study aimed to offer an enriched and thorough comprehension of the effect of childhood obesity on psychomotor behavior.

MATERIALS AND METHODS

This study used a literature search strategy to identify and acquire scholarly publications that were deemed crucial to the subject under study. A thorough investigation was conducted on scientific databases using relevant keywords in the search engine queries. The approach used in the literature search for this systematic review was the PICO framework, which incorporated the components of population (P), intervention (I), comparator (C), and outcome (O). The use of the PICO framework in this systematic review assisted the authors in developing clear study questions, conducting direct literature searches, and improving comprehension. This study employed the PICO framework to examine the impact of childhood obesity on psychomotor behavior through a comparative analysis of groups of obese children and control groups of healthy children (Methley et al. 2014).

This systematic review adhered to the standards outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to further expand our knowledge regarding the effect of obesity on the health and developmental outcomes of children. The use of PRISMA guidelines ensured that research methods were carried out in a systematic and transparent manner, improving the quality and consistency of systematic review reporting. The information and data used in this study were systematically obtained from reputable databases, including PubMed, ScienceDirect, and the Cochrane Library. The keywords explored in the literature search were ("effect") AND ("obesity") OR ("obesity in children") OR ("children with obesity") AND ("children") OR (adolescent) AND ("psychomotor") AND ("behavior"). We excluded studies that investigated either adults or people of higher ages, as well as those that did not provide findings related to psychomotor behavior. This systematic review

implemented solid inclusion criteria, specifying that all selected papers must be written in the English language, to ensure the consistency and convenience of data extraction and analysis. The age range of children included in this systematic review was limited to individuals between the ages of 5 and 14 years old. This specific age range was set to focus on a particular developmental stage and gain a comprehensive understanding of the effect of obesity on this age group. This systematic review exclusively contained original research articles to assure the inclusion of comprehensive and reliable research. The researchers made the decision to incorporate primary research and comprehensive evidence synthesis methodologies in the conduct of this study (Page et al. 2021).

The appropriate data from the selected studies were carefully and methodically collected. The dataset contained details pertaining to the population, design, and methodology of the studies. Additional information and data were obtained from several scholarly sources, such as scientific reports and books, by conducting searches on Google Scholar, targeted websites, and e-book platforms. The data were then classified and analyzed to draw conclusions regarding the relationship between obesity and psychomotor disorders in children. Moreover, the significant information from each study was gathered and organized using the software Zotero for Windows, version 6.0. The utilization of Zotero in this systematic review assisted the authors in efficiently managing, organizing, and citing references (Ivey & Crum 2018).

RESULTS

The literature search resulted in a total of 815 publications, as shown in Figure 1. The 815 publications were subjected to a rigorous screening process aimed at eliminating duplicates as well as papers with titles and abstracts that were deemed irrelevant. Afterwards, a total of 50 articles were screened for the purpose of determining their eligibility with regard to the main topic of this study. After a thorough evaluation of the titles and abstracts, 41 studies were deemed ineligible for inclusion in the systematic review. Among the 41 publications that were omitted, 39 had irrelevant topics, while one study was written in a non-English language. The remaining nine studies underwent further scrutiny, resulting in the exclusion of four publications on account of their undesirable results. As a result, a total of five studies were eligible for inclusion in this systematic review.

This systematic review included five studies that were conducted in Italy, Spain, Croatia, and Britain. The studies that were included in the analysis exhibited a wide range of sample sizes. The age range of the subjects examined in these studies was around 5 to 14 years old, as shown in Table 1. In this study, an assessment of the risk of bias was conducted to ensure an accurate review. This facilitated the researchers in proving the transparency of the findings derived from the selected studies during evidence synthesis. According to the findings presented in Table 2, the selected studies indicated a statistically significant relationship between childhood obesity and motor activity, with $p<0.05$.

Obesity has been known as a significant factor that causes a variety of diseases and disorders in both adults and children. In recent times, researchers have expressed a growing interest in investigating the potential relationship between obesity and the cognitive and psychomotor abilities of children. The study conducted by Gonzalez et al. (2020) revealed that obesity was linked with both agility and cardiorespiratory fitness. Children who were classified as obese or overweight also showed poor social interactions and relationships. This might be due to several factors related to the children's weight, such as low self-esteem. Obese children might have an increased risk of emotional problems that persist into adulthood. The empirical evidence indicated that obese girls experienced poorer outcomes in their social relationships. Consequently, they might be inclined to engage in solitary activities, including sedentary behavior, which could hinder their psychomotor development and efforts to reduce obesity (Black et al. 2015).

The study conducted by Madrona et al. (2019) examined the relationship between childhood obesity and psychomotor skills. The study involved a sample of 694 children aged 5 years who were enrolled in their third year of early childhood education in Spain in the year 2016. The sample was comprised of 46.7% girls and 53.3% boys. The findings of the study indicated that girls who were obese or overweight had poorer performance in motor activities, social relationships, and perceptual skills in comparison to girls who had a normal weight. In the study conducted by Marmeleira et al. (2017), a sample of 156 participants aged 6–10 years was examined to investigate the relationship between motor skills and body fat percentage in children. The findings revealed a negative correlation between motor skills and body fat percentage, indicating that higher levels of body fat were associated with poorer motor skills. Moreover, the study demonstrated that children who were within the normal weight range exhibited superior motor skills compared to their overweight or obese counterparts. It was observed that the negative impact of excessive body weight was more pronounced on gross motor skills, while fine motor skills appeared to be less influenced by the

constraints imposed by excessive body weight. According to the findings of Favieri et al. (2021), a significant correlation was observed between diminished motor skills and a higher BMI. The correlation was found to be influenced by food intake and eating behavior. Chagas & Batista (2016) revealed that overweight or obese adolescents had poorer motor coordination in comparison to their normal-weight counterparts. This observation was made regardless of the research subjects' levels of physical activity. Furthermore, Cheng et al. (2016) reported that a higher body weight was associated with decreased performance in motor skills, particularly in total and gross motor skills. These skills generally improve as individuals grow older.

A series of tests were conducted on girls and boys aged five years to assess the performance of their psychomotor skills. The tests consisted of a range of psychomotor variables, including physical, sensory, and social-emotional movements. The children were classified into different BMI groups, i.e., malnutrition, moderate malnutrition, average weight, overweight, and obesity. According to the results of more than half of the physical and motor skill tests, overweight girls had lower scores in comparison to their normal-weight counterparts. These results were apparent in regards to their dynamic adjustment, athletic performance, and balance. Simultaneously, their assessment of tonic attitude control yielded poor scores (Madrona et al. 2019).

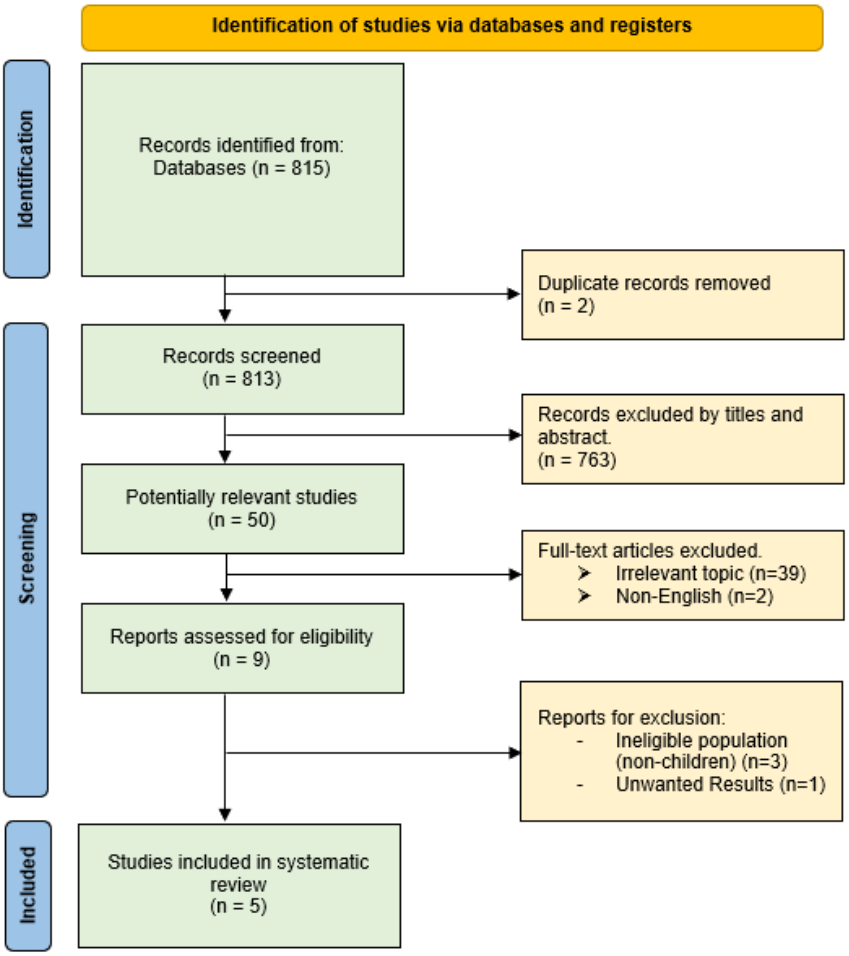

Figure 1. PRISMA flow diagram for this systematic review.

Table 1. Characteristics of the selected studies in this systematic review.

Authors, year	n	Age	Measurement instruments	Outcomes	Results
Madrona et al. (2019)	694	5	CPA	BMI, CPA	Obesity and overweight contributed to poorer performances in motor activities, social relationships, and perceptual skills.
Marmeleira et al. (2017)	156	6-10	Anthropometry measurement, BOTMP-SF	BF%, BMI	There was a negative correlation between children's motor skills and their body fat percentage. Normal-weight children had superior motor skills to those who are overweight or obese.
Favieri et al. (2021)	166 (96 childrens, 70 adolescents)	7-11, 15-18	Assessment of executive functions, Pearson's r correlation	BMI	There was a correlation between poorer motor inhibition and a higher BMI.
Chagas & Batista (2016)	56	13-14	PAQ-C, KTK	Levels of physical activity and motor skills, BMI	Overweight or obese adolescents had lower motor coordination compared to their normal-weight peers, regardless of their physical activity levels.
Cheng et al. (2016)	668	5, 10	BOTMP-SF, CDC standard measurements	BMI, motor skills	Higher weight was associated with decreased activity and motor skills.

Notes: CPA= Checklist of Psychomotor Activities; BOTMP-SF=The Bruininks-Oseretsky Test of Motor Proficiency-Short Form; PAQ-C=The Physical Activity Questionnaire for Older Children; KTK=*Körperkoordinationstest für Kinder* (Body Coordination Test for Children); CDC=Centers for Disease Control and Prevention; BF%=Body fat percentage.

Table 2. Detailed information on the selected studies' objectives and results.

Author, year	Objectives	Results
Madrona et al. (2019)	Assessment of children's psychomotor development using the CPA	There was a correlation between obesity and decreased performance in motor tasks (p=0.001).
Marmeleira et al. (2017)	Assessment of body composition through BMI and skinfold thickness measurement by using the BOTMP-SF	Obese children performed substantially poorer in gross motor abilities and general motor proficiency than children of normal weight (p<0.01).
Favieri et al. (2021)	Correlation analysis of executive functions and BMI	Greater BMI was associated with poorer set-shifting during adolescence and poorer motor inhibition during the initial stages of teenhood (p=0.001 in the BMI to age ratio based on performance).
Chagas & Batista (2016)	Assessment of physical activity and motor coordination levels using the PAQ-C and KTK	Adolescents who were classified as overweight or obese showed lower levels of motor coordination than their normal-weight peers (p<0.05). There was a significant correlation between physical activity and motor coordination observed in the normal weight group.
Cheng et al. (2016)	Assessment of children's motor skills using BOTMP-SF	A greater BMI was associated with severe motor abilities among children aged 5 years (p<0.001).

Notes: CPA= Checklist of Psychomotor Activities; BOTMP-SF=The Bruininks-Oseretsky Test of Motor Proficiency-Short Form; PAQ-C=The Physical Activity Questionnaire for Older Children; KTK=*Körperkoordinationstest für Kinder* (Body Coordination Test for Children).

DISCUSSION

In this study, we investigated the effect of childhood obesity, which can have significant adverse consequences because childhood is an important stage of human development. One of the consequences of obesity in children is psychomotor disorder. Moreover, physical exercise, basic movement abilities, and motor coordination are all related to childhood obesity (Han et al. 2018, Kliegman & III 2019). The coordinated movements involved in walking and throwing, which primarily involve larger muscle groups such as the upper body and legs, are commonly known as gross motor skills. Numerous studies have found a correlation between higher BMI and a subsequent delay in the development of children's fine and gross motor skills (Zacks et al. 2021).

Numerous studies have been conducted to investigate the correlation between obesity and psychomotor disorders in children. One of the studies revealed that there was a negative correlation between motor competency levels and BMI in

children and adolescents due to biomechanical problems. These problems might arise due to the increased mechanical effort required when performing motor activities that call for body weight support, particularly in individuals with higher body mass. The instruments used for the assessment of motor coordination in the study were the Physical Activity Questionnaire for Older Children (PAQ-C) and the Body Coordination Test for Children (Körperkoordinationstest für Kinder/KTK). The PAQ-C assesses the general levels of physical activity of participants, while the KTK is a comprehensive test that evaluates various aspects, including balance, rhythm, strength, laterality, speed, and agility (Chagas & Batista 2016).

Evidence from multiple studies demonstrated that BMI strongly influenced the performance of physical-motor skills. Assessment using the Bruininks-Oseretsky Test of Motor Proficiency-Short Form (BOTMP-SF) was conducted to evaluate both the gross and fine motor abilities of 156 children. The BOTMP-SF assesses gross motor competence (i.e., running speed and agility, balance, bilateral coordination, and strength), gross and fine motor competence (i.e., upper limb coordination), and fine motor development (i.e., response speed, visual motor control, and upper limb speed and dexterity). The results showed that gross motor skills were more adversely affected by extra body weight than fine motor skills (Marmeleira et al. 2017, Martins et al. 2022).

According to the analyses of the selected studies, obese girls perform poorer than their peers in terms of dynamic coordination, motor function, and balance. The Checklist of Psychomotor Activities (CPA) was utilized to examine 694 obese children pertaining to various variables, such as postural alterations and decreased stability. The results showed that boys had higher motor performance than girls. The Checklist of Psychomotor Activities (CPA) is a tool for assessing the psychomotor development of children. It consists of three domains: physical-motor (i.e., laterality, dynamic coordination, balance, motor execution, and tonic postural control), perceptual (i.e., respiratory control, schema and body image, motor dissociation, visual-motor coordination, and spatial orientation), and social-emotional (i.e., control and social relations). In the study, girls had lower levels of physical activity and motor experiences compared to boys, indicating a substantial impact of BMI on their psychomotor capacity (Madrona et al. 2019). These results might be attributable to the boys' greater involvement in physical activity compared to girls. Poorer set-shifting in adolescence and poorer motor inhibition in young adulthood were found to be related to a higher BMI.

In addition, compared to babies and toddlers with lower fat deposits under the skin, those with more subcutaneous fat had a higher incidence of motor problems, including delayed developmental milestones of rolling over, sitting up, and crawling (Cataldo et al. 2016). According to Favieri et al. (2021), executive functioning difficulties were reported to be related to a higher BMI throughout adolescence and young adulthood. This observation took place among a healthy sample that represented the majority of the population in these age groups, with the subjects falling within the healthy weight to overweight range. In another recent study conducted by Martins et al. (2021), there was a correlation between obesity and several negative outcomes, including impaired motor skill development and a worsened quality of life. This was evidenced by the obese individuals' lower scores in the areas of emotional well-being and physical.

Strength and limitations

The study used a systematic review approach by initially conducting a rigorous and comprehensive search of the current literature. This implies that a wide range of relevant studies were screened for inclusion, improving the validity and reliability of the results. This systematic review ensured the accuracy and validity of the information acquired in evaluating the psychomotor activity of children. Additionally, it featured control groups of healthy children, allowing for a direct comparison of the psychomotor activity of obese and non-obese children. Therefore, it became easier to pinpoint how obesity specifically affects psychomotor performance.

It is noteworthy to acknowledge the limitations of this systematic review study. First, different methodologies were employed for each of the selected studies. Second, the age range of the research subjects was rather broad, and the instruments were diverse across studies. Third, two out of five studies produced different results compared to the remaining studies. It was challenging to establish a gold standard for evaluating childhood obesity and associated psychomotor side effects due to the various outcomes of the selected studies. Several studies revealed that there was no negative correlation between obesity and motor activity.

CONCLUSION

A high body mass index (BMI), either classified as overweight or obese, has an effect on the psychomotor behavior of children. Children who are classified as obese or overweight have a high probability of experiencing a decline in their

psychomotor behavior. The suboptimal psychomotor performance observed in overweight and obese children is attributable to their poor body control and balance, which commonly inhibit their ability to move effectively.

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Conflict of interest

None.

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None.

Author contribution

MAK contributed to the conception and design, drafting of the article, analysis and interpretation of the data, and collection and assembly of the data. PSR, S, and NEFMF contributed to the conception and design, drafting of the article, critical revision of the article for important intellectual content, and final approval of the article.

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All words in Latin must be written in italics. The use of abbreviations is generally agreed upon, and an extension must be given in the first mention of the abbreviation. Decimal numbers are marked with points (.).

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The total number of tables and figures are limited, advisably not more than five, and should be cited in ascending Arabic numerals according to their first appearance in the manuscript. All tables and figures should be cited in the body text of the manuscript.

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References

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Rose ME, Huerbin MB, Melick J, et al (2002). Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res 935, 40-46. doi: ……

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Reece JB, Lisa AU, Peter VM, et al (2010). Campbell biology. Pearson, London.

Chapter in a book

Meltzer PS, Kallioniemi A, Trent JM (2002). Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW (eds). The genetic basis of human cancer, New York, McGraw-Hill,

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Darwin C. On the origin of species by means of natural selection or the preservation of favoured aces in the struggle for life [Internet]. London: John Murray; 1859. Chapter 5, Laws of variation. [cited 2010 Apr 22]. Available from: http://www.talkorigins.org/faqs/origin/chapter5.html.

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Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

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Kay JG. Intracellular cytokine trafficking and phagocytosis in macrophages [dissertation]. St Lucia, Qld: University of Queensland; 2007

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Pahl KM. Preventing anxiety and promoting social and emotional strength in early childhood: an investigation of risk factors [dissertation on the Internet]. St Lucia, Qld: University of Queensland; 2009 [cited 2017 Nov 22]. Available from: https://espace.library.uq.edu.au/view/UQ:178027.

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