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Effectiveness of butterfly pea (*Clitoria ternatea*) flower extract as an adjuvant therapy for the treatment of pulmonary tuberculosis patients

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ABSTRACT

Introduction: Tuberculosis (TB) is the main cause of infectious death. Butterfly pea flower therapy could potentially improve the expression of inflammatory cytokines in patients undergoing TB treatment. This study aimed to evaluate the success of adjuvant butterfly pea flower therapy in TB patients.

Methods: A total of 28 TB patients who met the inclusion and exclusion criteria were divided into (1) the anti-TB plus butterfly pea flower group and (2) the anti-TB group, consisting of rifampicin, isoniazid, pyrazinamide, and ethambutol. Analysis of the interferon-γ (IFN-γ) and interleukin-10 (IL-10) cytokines was carried out using the enzyme-linked immunosorbent assay. The profiles of hemoglobin (Hb), leukocytes, platelets, hematocrit (Ht), granulocytes, and erythrocyte sedimentation rate (ESR) were analyzed using a hematology analyzer. Microscopic analysis of *Mycobacterium tuberculosis* was carried out by Ziehl-Nielsen staining. Evaluation of clinical symptoms included fever, cough, shortness of breath and jaundice.

Results: Administration of adjuvant butterfly pea flower therapy to anti-TB therapy increased the pro-inflammatory IFN-γ compared to anti-TB therapy (1.03 ± 0.82 vs 0.49 ± 0.39 pg/mL; $P<0.05$), the anti-inflammatory IL-10 increased insignificantly (1.16 ± 0.96 vs 0.78 ± 0.79 pg/mL; $P>0.05$). Adjuvant of butterfly pea flower also provided statistically significant benefits in reducing leukocytes, platelets, and acid-fast bacilli (AFBs) and improving the symptoms of cough and shortness of breath ($P>0.05$). This adjuvant therapy did not cause side effects.

Conclusion: Adjuvant butterfly pea flowers may provide benefits for TB patients under anti-TB therapy. Further research is still needed to confirm the adjuvant effect of butterfly pea flower on a large number of TB patients.

Implication for health policy/practice/research/medical education:
This study provides novel information regarding the effect of adjuvant butterfly pea flower therapy for TB patients in the first 2 months of initiation phase treatment. Butterfly pea flowers affect changes in inflammatory cytokines, improve leukocyte and platelet parameters, and improve patient clinical symptoms. This article also presents the preparation method of butterfly pea flower extract and its phytochemical content for further research guidelines.

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Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is a serious infection problem in the world. TB is still the main cause of death due to infection, and currently, no country is free from this problem (1). TB cases in Indonesia tend to increase every year. The cure rate is not optimal; for example in 2022, the TB cure rate in Central Kalimantan Indonesia was only 82.9% of cases. Treatment coverage in Central Kalimantan is below 50% and the number of drug-resistant TB is estimated at 24% of all cases. The Pangkalan Bun region is one of those that contributes the most TB cases in Central Kalimantan Indonesia (2).

The success of pulmonary TB treatment depends on

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patient compliance, regularity of treatment, and also routine physical and laboratory examinations such as cytokines interferon-γ (IFN-γ) and interleukin-10 (IL-10) which are associated with inflammation (3,4). Interferon-γ plays a role in protective immunity against *M. tuberculosis* infection by maintaining the bacteriostatic function of macrophages. The role of IFN-γ can be inhibited by the activity of the T-helper 2 (Th2) component. Active TB patients are reported to experience increased IL-10 production and risk of damage due to immunopathological mechanisms. IL-10 has the function of negatively regulating the immune system by inhibiting macrophage function, therefore neutralization of IL-10 is expected to increase IFN-γ and the ability of macrophages to kill *M. tuberculosis* (5). A study on TB patients stated that treatment using TB drugs for 2 months reduced both cytokine levels IFN-γ and IL-10 (6).

TB patients can also experience abnormalities in the hematological profile which may occur due to complications from using anti-TB drugs. Increased leukocytes associated with lymphocytosis and neutrophilia are often found in advanced TB patients. The erythrocyte sedimentation rate (ESR) decreases at initial diagnosis but may increase after treatment is completed due to the inflammatory process, while platelet levels may remain decreased (7). TB patients are also at risk of experiencing a decrease in hemoglobin (Hb) and hematocrit (Ht) due to the release of inflammatory cytokines by immune cells in response to infection (8).

Natural medicines can be used as adjuvant therapy for the treatment of TB. Butterfly pea flowers (*Clitoria ternatea*) have been widely studied for their benefits in controlling blood sugar, as well as being anti-cancer and anti-inflammatory (9,10). Butterfly pea flower extract contains bioactive components such as flavonol glycosides, flavones, phenolic acids, terpenoids, alkaloids, and anthocyanin compounds (11,12). Anthocyanins are thought to play a determining role in maintaining immune cytokines and anti-inflammatory agents by inhibiting prostaglandin synthesis through the role of the cyclooxygenase enzyme (13).

There is a report that butterfly pea flower extract might be beneficial to TB patients (14). Butterfly pea flower extract given for a month to patients with *Mycobacterium leprae* infection was also declared safe and had an anti-inflammatory effect by increasing the patient's cortisol levels (15).

Based on the previous explanation, butterfly pea flower extract can be optimized as adjuvant therapy for TB patients to suppress the expression of pro-inflammatory cytokines and increase the expression of anti-inflammatory cytokines. This research is novel to assess the therapeutic efficacy of administering adjuvant butterfly pea flower therapy to pulmonary TB patients. The success of therapy was evaluated from the results of IFN-γ, IL-10, hematological profiles, microscopic profiles of acid-fast bacilli (AFBs), and the clinical symptoms of TB patients after 2 months of treatment. The butterfly pea flower extract used in this research was made using the decocta method, a simple extraction method, which fulfils hygiene and dose standardization (16).

Materials and Methods

Study design and ethical approval

This study was a prospective randomized clinical trial, pre-post with control design, single-blind, multiple centers in four Community Health Centers in the Pangkalan Bun region (Mendawai, Madurejo, Arsel, and Pelingkau Community Health Centers) and centered at the Sultan Imanuddin Pangkalan Bun Regional General Hospital. The research was carried out in May–July 2024 and received ethical approval from the Ethics Commission at the Faculty of Medicine, Universitas Sultan Agung Semarang Indonesia with number 90/III/2024/Komisi Bioetik. This research received ethical approval from the Ethics Commission at the Faculty of Medicine, Universitas Sultan Agung Semarang Indonesia with number 90/III/2024/Komisi Bioetik. The herbal medicine whose effectiveness was tested in this study as an adjuvant therapy for pulmonary TB patients was decocta extract of *Clitoria ternatea* flower (Fabaceae; butterfly pea flower). The trial was registered to "ClinicalTrials.gov" with ID number NCT06794502.

Determination of research subjects

The subjects were selected based on the following inclusion criteria: 1) Pulmonary TB patients were positive based on the results of the Rapid Molecular Test GeneXpert *M. tuberculosis* (MTB)/rifampicin (RIF), 2) Patients who were undergoing the initiation phase of anti-TB treatment for the first time, 3) Non-multi drug resistant (MDR)/extensively-drug resistant (XDR) TB patients with first-line anti-TB treatment (rifampicin, isoniazid, pyrazinamide, and ethambutol), 4) Patients >18 years old, 5) Patients who were willing to become research subjects and signed informed consent. The exclusion criteria were: 1) TB patients with human immunodeficiency virus (HIV) positive, 2) Patients who did not complete the study, 3) Patients with non-compliance in taking medication, 4) Patients with comorbidities (autoimmune and cancer), 5) Smoking patients, and 6) Patients contraindicated in using butterfly pea flower extract. Patients were divided into two treatment groups by random sampling. Group 1 was patients who used anti-TB drugs plus butterfly pea flower, while group 2 was patients who used anti-TB drugs (control group). Random sampling was carried out by tossing a coin. The first open coin determined which subject was included in group 1, then the second subject who met the inclusion criteria was put into group 2, and so on until each group had 15 research subjects.

Preparation of butterfly pea flower decocta extract

Butterfly pea flower extract was made using the decoction method with boiling at 100 °C for 10 minutes (16). The single dose of butterfly pea flower for patients was 5 dried flowers (boiled with 40 mL of water), which was known to be effective for thinning phlegm in an in vitro study (17). The stability test of butterfly pea flower decocta was observed for 3-30 days in a chiller and at room temperature based on changes in colour, smell, and taste.

Drug treatment of research subjects

The adjuvant therapy group of TB patients (group 1) was given an anti-TB regimen and butterfly pea flower extract of 40 mL/d. The patients in the anti-TB monotherapy group (group 2) were given a regimen consisting of rifampicin, isoniazid, pyrazinamide, and ethambutol. Anti-TB drugs and butterfly pea flower extract were administered during the initiation phase of treatment for 2 months. Determining the dosage of anti-TB drugs and monitoring the use of butterfly pea flower decoct extract were carried out by doctors at the Community Health Center where the patients were receiving treatments. Monitoring patients for medication compliance was carried out by researchers and medical officers (drug swallowing supervisors).

Measurement of total flavonoid content (TFC) and total phenolic content (TPC) of butterfly pea flower extract

For TFC measurements, a flavonoid standard curve was prepared using quercetin (Sigma Adrich, concentration of 0-100 µg/mL). A solution of 1 mL of butterfly pea flower decocta extract was added to 0.2 mL $AlCl_3$ 10% (b/v) (Merck) in methanol (Merck). Next, 0.2 mL of CH_3COONa 1 M (Merck) and 5.6 mL of distilled water were added to the solution and homogenized and incubated for 15 minutes. The absorbance of the entire mixed solution sample was read using a spectrophotometer with a wavelength of 425 nm (18). For TPC measurements, 0.1 mL of butterfly pea flower extract solution was added to 0.1 mL of Folin-Ciocalteu 50% (Sigma Aldrich) and vortexed. Next, 2 mL of 2% Na2CO3 solution (Merck) was added and vortexed, then incubated for 30 minutes in a dark room. Absorbance was read at a wavelength of 750 nm using a spectrophotometer. A calibration curve was prepared using gallic acid (Sigma Aldrich) as a standard. TPC was expressed as mg gallic acid equivalents/g (mgEQ/g) sample) (19).

Screening of IFN-γ, IL-10, and hematological profiles

Serum samples were taken from the patient's arm vein blood. Examination of IFN-γ and IL-10 levels was carried out using the method according to the kit (EliKine™ Human IFN-γ/IL-10 ELISA Kit 48 wells). A total of 100 µL of standard solution and patient serum samples were incubated for 2 hours at room temperature, then washed with 250 µL of 1×Wash Buffer. As much as 100 µL of 1×Human IFN-γ/IL-10 detection antibody was added to the sample well and incubated for 1 hour at room temperature. After washing, 100 µL 1×Streptavidin-HRP was added to the wells and then incubated for 30 minutes at room temperature. Next, 100 µL of HRP substrate solution (TMB) was added to the well and then incubated for 15 minutes at room temperature. The stop reaction was carried out by adding 50 µL of stop solution until the colour of the solution changed from blue to yellow. The absorption of colour changes was read within 30 minutes using a wavelength of 450 nm. For hematology examination, the blood sample plus EDTA (Merck) was homogenized then the parameters of Hb, Ht, leukocytes, lymphocytes, neutrophils, platelets, and ESR were checked using a hematology analyzer (Sysmex XP-300).

Evaluation of the microscopic examination of acid-fast bacilli (AFBs) and the patient's clinical symptoms

For AFB examination before and after treatment, thick and purulent sputum was taken from the patient using a stick and spread on a glass object, then fixed over alcohol for 3-5 seconds. Staining began by pouring carbol fuchsin (Sigma Aldrich) until it covered the entire surface of the glass object, then heated carefully until steam came out (not boiling). After drying, 3% alcohol acid was added to the glass object until the red colour of the fuchsin disappeared and then washed with running water. Next, the methylene blue solution (IndoReagent) was poured into the glass object and left for 10-20 seconds, then washed with running water. Once dried, it was examined under a microscope with 1000x magnification (20). After completion of treatment, several clinical parameters related to treatment success were evaluated, including fever, cough frequency, symptoms of shortness of breath, and the risk of jaundice due to drug use.

Statistical analysis

The IFN-γ, IL-10, and hematological profile values were first tested for normality using Kolmogorov-Smirnov and homogeneity testing with Shapiro-Wilk. A comparison of the mean changes in cytokine values and hematological profiles of the two groups was analysed by independent t-test. Microscopic differences in AFB patients were described descriptively. Evaluation of the clinical symptoms before and after treatment were analysed using Chi-squared test. The results of microscopic readings were described in the scanty category (found 1-9 AFBs/100 field of view-FoV), 1+ (10 -99 AFBs/100 FoV, 2+ (1-10 AFBs/1 FoV), dan 3+ (≥ 10 AFBs/1 FoV) (20).

Results

Distribution of research subject patient groups

A total of 30 patients diagnosed with TB using the rapid molecular test and met the inclusion and exclusion criteria

were divided into 2 treatment groups, 1) the anti-TB plus butterfly pea flower group and 2) the anti-TB monotherapy group. In both groups, some individual patients were excluded from this study because incompliance in taking medication regularly and having difficulty in monitoring treatment (Figure 1).

Preparation of butterfly pea flower decocta extract and stability test

The butterfly pea flower decocta extract stored at cold temperatures (2-6 °C) did not experience changes in quality as measured by smell, taste, and color. When the storage was carried out at room temperature (28-30 °C), the taste changed to sour, the smell became rancid and the color oxidized after 3 days of storage.

Demographic characteristics of patients

Subject characteristics were described based on age, gender, and educational background. The research results showed that individuals aged 20-45 years and the men subjects had a higher tendency to contract TB disease (Table 1).

Table 1. Frequency distribution of characteristics of tuberculosis (TB) patients based on age, gender, and educational background

Patient's characteristic	Type of treatment group	
	Anti-TB & adjuvant	Anti-TB
Age (y)		
20-44	9	8
45-59	2	5
60-69	3	1
Gender		
Male	8	8
Female	6	6
Educational background		
High school	9	10
Diploma or higher	5	4

Evaluation of IFN-γ and IL-10 cytokines

Evaluation of the success of anti-TB therapy and anti-TB plus adjuvant butterfly pea flower therapy was carried out after completion of the 2-month initiation phase of treatment. For the inflammatory cytokine profile, the change factor analysis (CFA) for each parameter and treatment group was calculated using the formula:

$$CFA = \frac{\text{IFN-}\gamma \text{ or IL-10 pg/mL after treatment-value before treatment}}{\text{IFN-}\gamma \text{ or IL-10 pg/mL value before treatment}}$$

The CFA values of the IFN-γ or IL-10 were compared between the anti-TB and anti-TB plus adjuvant. Based on the results of the independent samples t-test, there was a significant increase in IFN-γ levels in the anti-TB plus adjuvant therapy group compared to the anti-TB group. Giving butterfly pea flowers did not provide a significant difference in benefits for increasing IL-10 compared to anti-TB therapy (Table 2).

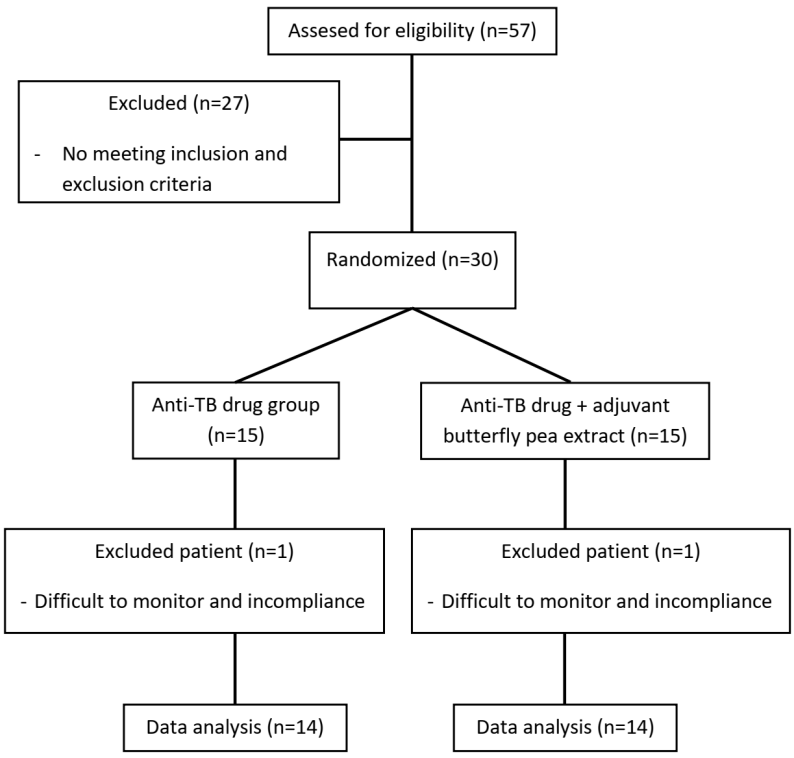

Figure 1. Flowchart of selecting eligible TB patients, randomization, and determining the number of patients analyzed.

Table 2. Changes in interferon-γ (IFN-γ) and interleukin-10 (IL-10) levels (pg/mL) after administering butterfly pea flower as an adjuvant to pulmonary tuberculosis (TB) patients

Parameter	Anti-TB & Adjuvant		Anti-TB		Changes		*P* values
	Pre	Post	Pre	Post	Anti-TB & adjuvant	Anti-TB	
IFN- γ	2.40 ± 0.96	4.64 ± 1.71	1.75 ± 0.51	2.48 ± 0.54	1.03 ± 0.82*	0.49 ± 0.39	0.027
IL-10	1.22 ± 0.53	2.23 ± 0.30	1.24 ± 0.59	1.85 ± 0.41	1.16 ± 0.96	0.78 ± 0.79	0.249

Data are presented as mean±Standard deviation. *Significantly different compared to the anti-TB group.

Hematology profiles evaluation

For hematological profile analysis, the change factor analysis (CFA) for each parameter, namely Hb (g/dL), leukocytes and platelets (cells/μL), hematocrit and granulocytes (%), and ESR (mm/hour) was calculated using the formula:

$$CFA = \frac{\text{Each parameter value after treatment-value before treatment}}{\text{Value before treatment}}$$

Administration of anti-TB therapy and adjuvant butterfly pea flower extract could significantly reduce leukocytes and platelets in TB patients when compared with anti-TB therapy (Table 3).

Acid-fast bacilli microscopic and clinical symptoms evaluation

Based on the results of the microscopic examination of AFBs, none of the patients who received adjuvant butterfly pea flower were assessed as positive +2 and +3, better than those in the anti-TB therapy group (Table 4). Meanwhile, based on the changes in clinical symptoms, there was no difference between the two groups. However, the frequency of coughing and asphyxiate occurred more frequently in patients given anti-TB therapy (Table 5).

Total flavonoid and phenolic contents of butterfly pea flower extract

TPC in the butterfly pea flower decocta extract (171.87 ± 2.02 μg/mL equivalent to gallic acid) was greater than total flavonoids (113.04 ± 12.22 μg/mL equivalent quercetin). Every day drink of 40 mL of butterfly pea flower extract through each patient is equal to 4.52 mg of flavonoids and 6.84 mg of phenols.

Discussion

In this study, adult male patients dominated the cases relevant to information from WHO in 2022, as many as 90% of those infected with TB would be adult men (1). The predominance of male patients can be caused by the high level of mobility which requires more energy; most of them are active smokers and alcohol drinkers which can

Table 3. Changes in the hematological parameters after administering butterfly pea flower as an adjuvant therapy to pulmonary tuberculosis (TB) patients

Parameters[a]	Anti-TB & adjuvant		Anti-TB drugs		Changes		*P* values
	Pre	Post	Pre	Post	Anti-TB & adjuvant	Anti-TB Drugs	
Haemoglobin	10.28 ± 1.24	13.01 ± 1.13	10.30 ± 1.85	12.35 ± 1.34	0.21 ± 0.10	0.23 ± 0.20	0.418
Leucocytes	8.78 ± 2.40	5.99 ± 1.61	9.05 ± 2.50	6.69 ± 1.98	- 0.50 ± 0.33*	- 0.25 ± 0.17	0.005
Platelets	359.53 ± 101.96	278.60 ± 88.37	311.13 ± 95.84	277.07 ± 67.50	- 0.17 ± 0.41*	- 0.06 ± 0.27	0.036
Hematocrit	30.35 ± 4.27	37.97 ± 2.86	31.18 ± 6.15	36.51 ± 3.61	0.27 ± 0.17	0.20 ± 0.23	0.105
Granulocytes	70.07 ± 8.99	58.93 ± 9.79	65.87 ± 13.69	63.87 ± 16.16	- 0.15 ± 0.14	- 0.03 ± 0.48	0.361
ESR	45.73 ± 16.24	23.60 ± 10.39	42.00 ± 18.41	20.67 ± 9.36	- 0.49 ± 0.11	- 0.50 ± 0.10	0.678

[a] Haemoglobin: g/dL; leucocytes and thrombocytes: cells/μL; hematocrit and granulocytes: %; ESR: mm/h. .*Significantly different compared to Anti-TB group.

Table 4. Number of pulmonary tuberculosis (TB) patients based on the results of acid-fast bacilli (AFBs) microscopic examination

Result of AFBs	Type of treatment			
	Anti-TB & adjuvant		Anti-TB	
	Pre	Post	Pre	Post
Negative	None	12	None	11
Positive +1	9	3	10	3
Positive +2	4	None	3	1
Positive +3	2	None	2	None

Table 5. The number of pulmonary tuberculosis (TB) patients with clinical symptoms based on the type of treatment

Symptoms	Type of treatment						*P* value
	Anti-TB & adjuvant			Anti-TB			
	None	Seldom	Often	None	Seldom	Often	
Fever	12	3	0	12	3	0	1.000
Cough with Phlegm	14	1	0	12	3	0	0.282
Asphyxiate	13	2	0	11	3	1	0.361
Jaudince	None of the subjects experienced jaundice						

reduce the body's immunity (21,22).

Normal levels of IFN-γ vary greatly, but one study stated that a range of 7-124 pg/mL was considered normal (23). In this study, although giving butterfly pea flowers significantly benefited TB patients in increasing IFN-γ, the average levels were still below the normal range (Table 2). Interferon-γ is a crucial cytokine in the process of eliminating bacteria in inflamed tissue and is very important in the mechanism of controlling pulmonary TB infection. Decreased IFN-γ levels also influence macrophage activation strategies in an effort to eliminate *M. tuberculosis* (24). Increased levels of IFN-γ can increase the risk of lung inflammation, so other cytokines such as IL-10 are needed to balance the chronic inflammation process (4).

The IL-10 level decrease early in TB infection. A study stated that IL-10 concentrations in TB patients were lower compared to healthy individuals. These findings indicate that the Th1 type immune response is more dominant in TB patients, indicating a protective response against *M. tuberculosis* infection. IL-10 is mainly produced by Th2 cells in response to *M. tuberculosis* infection but can suppress immune responses and limit tissue damage by inhibiting excessive inflammatory responses. Excessive production of IL-10 can control infection, thereby worsening the prognosis of TB disease (25), therefore, the value of IFN-γ/IL-10 ratio has been widely studied. A higher IFN-γ/IL-10 ratio value is considered more useful for assessing the prognosis or recovery of TB patients. If the ratio of the two cytokines is low, the risk of TB recurrence increases (26). In this study, the IFN-γ/IL-10 ratio was higher in the adjuvant butterfly pea flower therapy group. The benefits of butterfly pea flowers for inhibiting cytokine storms, including inhibiting other inflammatory cytokines such as IL-6, can be due to the anthocyanin compound ternatin (27,28).

TB can affect the hematopoietic system causing abnormalities in hematological parameters such as anemia, leukocytosis, and thrombocytosis (29). Leukocytes increase after *M. tuberculosis*–infection, which indicates an immune response to fight infection. Giving anti-TB therapy in the intensive phase is considered successful in reducing leukocyte levels in TB patients (30). In both treatment groups, leukocyte levels decreased significantly and were within the normal range. However, adjuvant administration of butterfly pea flower was statistically better for reducing leukocyte levels in TB patients (Table 3). This can be due to the ability of butterfly pea flowers as antibacterial and anti-*M. tuberculosis* (31,32). This study also stated that adjuvant butterfly pea flower therapy was able to significantly reduce platelets in TB patients compared to anti-TB therapy, although clinically the platelet levels in both groups were within the normal range (Table 3). Thrombocytosis usually occurs early in TB infection and can be an indication of significant inflammation due to *M. tuberculosis* infection; it is a parameter of the patient's response to treatment (33).

There were no significant differences in other hematological parameters between the two treatment groups. Anemia due to a decrease in Hb can occur before starting anti-TB treatment, as can Ht. Low Hb and Ht values in TB patients can be caused by erythrocyte destruction (34). In this study, adjuvant butterfly pea flower therapy did not increase Hb and Ht better than anti-TB monotherapy. In this study, there was a decrease in granulocyte and ESR after 2 months of treatment but there was no difference between the two treatment groups. Granulocytosis in TB patients is an inflammatory response to *M. tuberculosis* infection. A decrease in neutrophils accompanied by an increase in lymphocytes can be a good prognosis for assessing the success of TB therapy (35). An increase in ESR in TB patients is used to indicate acute/chronic tissue damage or inflammation. Inflammatory reactions can cause an increase in plasma viscosity and fibrinogen which can ultimately increase the ESR value (36). In this study, both groups of patients experienced a decrease in ESR levels until they reached normal values after finishing treatment.

Treatment evaluation using microscopic data showed that adjuvant butterfly pea flower therapy was better in reducing the positive status of the number of AFBs in sputum (Table 4). Based on the symptom data, patients who received adjuvant butterfly pea flower therapy still experienced fever but the number was lower than in the anti-TB drug monotherapy group. Butterfly pea flower is known to have antipyretic effects (10). Patients with adjuvant butterfly pea flower also experienced fewer symptoms of coughing up phlegm after completing treatment. This is in line with other research which states that butterfly pea flower tea has potential mucolytic activity

(17). A series of other studies have also been carried out to confirm the performance of butterfly pea flowers as an anti-asthmatic and cough suppressant (37,38). None of the patients experienced jaundice, indicating that anti-TB and adjuvant butterfly pea flower did not show hepatotoxic effects. Phenolics from butterfly pea flowers have been widely studied for their activity as hepatoprotectors in experimental animals (39).

The therapeutic effect of adjuvant butterfly pea flower in TB patients can be caused by its phenolic and flavonoid contents. The TFC content of butterfly pea flower water extract can reach 6 to 78 mg/mL, while the TPC can reach 0.27 to 233 mg/mL. Decocta extract using water in this study had lower levels of TFC and TPC, which can be caused by variations in the growing environment or drying factors (18, 40). Low levels of TFC and TPC are the reasons why adjuvant butterfly pea flower therapy cannot provide optimum effect for the treatment of TB patients, considering that the bioavailability of flavonoids and phenolics is also low in serum (41).

Adjuvant butterfly pea flower therapy may provide the prospect in successful treatment of pulmonary TB in several aspects related to inflammatory cytokines, hematological profile, microscopic AFBs profile, and clinical symptoms. This study had a limited sample size and placebo consumption by anti-TB therapy patients. However, taking large amounts of anti-TB drugs may reduce the effect of not taking a placebo. Furthermore, this study is a phase 1 clinical trial, then minimum of 20 subjects are usually considered to meet the test requirements (42). Further research is still needed to confirm the adjuvant effect of butterfly pea flowers on larger sample size TB patients, monitor variables that influence treatment, and evaluate the dose of butterfly pea flower extract used.

Conclusion

Adjuvant butterfly pea flower decocta extract for anti-TB treatment during the 2-month treatment phase provided significant benefits in increasing IFN-γ and reducing blood leukocyte and platelet levels. Providing adjuvant therapy also provided benefits in terms of the number of AFBs on microscopic examination and reduced the frequency of clinical symptoms of cough with phlegm and shortness of breath. All treatments were considered safe based on the patient's clinical profile. Therefore, this adjuvant therapy might be beneficial in these patients.

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Authors' contribution

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Funding acquisition: Maya Dian Rakhmawatie.
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Methodology: Maya Dian Rakhmawatie, Sri Darmawati, Jassanti.
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Software: Maya Dian Rakhmawatie, Jassanti.
Supervision: Sri Darmawati.
Validation: Sri Darmawati.
Visualization: Maya Dian Rakhmawatie.
Writing–original draft: Maya Dian Rakhmawatie, Sri Darmawati, Jassanti.
Writing–review & editing: Maya Dian Rakhmawatie.

Conflict of interests

The authors declare that they have no conflict of interest.

Ethical considerations

The authors have paid close attention to ethical considerations concerning authorship, data collection, review, and analysis. This research has received ethical approval from the Ethics Commission at the Faculty of Medicine, Universitas Sultan Agung Semarang Indonesia with number 90/III/2024/Komisi Bioetik. The trial was registered to the ClinicalTrials.gov with ID number NCT06794502, with the following link https://clinicaltrials.gov/study/NCT06794502.

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