Original Article

Effect of Spinach Extract Supplementation Combined with Iron Tablets on Hemoglobin Concentration among Pregnant Women: A Quasi-Experimental Study

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ABSTRACT

Background: Anemia during pregnancy remains a significant maternal health problem, primarily associated with reduced hemoglobin concentration. Although iron tablet supplementation is a standard intervention in antenatal care, its effectiveness may be enhanced through complementary nutritional strategies. However, limited evidence exists regarding the effectiveness of spinach extract supplementation combined with iron tablets as an adjunct to routine iron supplementation among pregnant women. Therefore, this study aimed to evaluate the effect of spinach extract supplementation on hemoglobin concentration in pregnant women.
Methods: This quasi-experimental pretest–posttest two-group study with a control group was reported in accordance with the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) guideline. A total of 60 pregnant women were selected using purposive sampling and allocated to either the intervention or control group through nonrandom assignment based on existing antenatal care groupings. The intervention group received spinach extract supplementation combined with iron tablets combined with iron tablets, while the control group received iron tablets only for 14 days. Hemoglobin concentration was measured at baseline and after the intervention using an EasyTouch GCHb hemoglobin meter. Data were analyzed using paired-samples t-tests and independent-samples t-tests.
Results: Hemoglobin concentration increased significantly in both groups after the intervention. At baseline, there was no significant difference in mean hemoglobin concentration between the intervention and control groups. After the intervention, the intervention group had a significantly higher mean hemoglobin concentration than the control group. The mean hemoglobin change was 1.12 ± 0.32 g/dL in the intervention group and 0.76 ± 0.33 g/dL in the control group. The between-group difference in hemoglobin change was 0.36 g/dL (95% CI: 0.19–0.53; $p < 0.001$), with a large effect size (Cohen's $d = 1.11$).
Conclusion: Spinach extract supplementation combined with iron tablets may provide an effective complementary strategy for improving hemoglobin concentration among pregnant women. However, further randomized studies with larger sample sizes and longer follow-up periods are recommended to confirm these findings.

Keywords: Anemia; Hemoglobins; Pregnancy; Spinacia; Diaetry Supplements.

Implications for Practice:

- Spinach extract supplementation may be incorporated as an evidence-based complementary nutritional intervention to support anemia prevention and hemoglobin monitoring during routine antenatal care.
- Maternal health services may consider updating nutrition counseling protocols and clinical guidelines by integrating culturally acceptable and affordable plant-based supplementation strategies.
- Health policymakers and professional training programs may use these findings to develop responsive maternal nutrition interventions that can be adapted to local resources and community needs

Introduction

Anemia during pregnancy remains a critical maternal health problem because it can compromise maternal well-being, fetal development, and pregnancy outcomes (O'Toole et al., 2024). As a condition characterized by reduced hemoglobin (hb) concentration, anemia may impair oxygen transport to maternal and fetal tissues, thereby increasing the risk of fatigue, reduced physical capacity, adverse birth outcomes, and pregnancy-related complications (Finkelstein et al., 2024). This issue is closely aligned with the Sustainable Development Goals, particularly the targets related to maternal health, nutrition improvement, and reduction of preventable maternal and neonatal morbidity and mortality (Rahman et al., 2022).

Globally, anemia continues to affect vulnerable populations, including pregnant women, women of reproductive age, adolescent girls, and young children (Rahman et al., 2022). The World Health Organization estimates that anemia affects approximately 37% of pregnant women worldwide, indicating that it remains a major public health concern (WHO, 2025). In Indonesia, anemia among pregnant women also remains a priority maternal health issue. National data reported that 27.7% of pregnant women experienced anemia, while local health records showed anemia prevalence among pregnant women in Baleendah Primary Health Care Center, Bandung Regency, reached 4.56% in 2023 (Ministry of Health of the Republic of Indonesia, 2023). These figures indicate that anemia prevention and management require continued attention from both clinical and community health perspectives.

Iron deficiency is recognized as one of the most common causes of anemia during pregnancy (Benson et al., 2021). Physiological changes during pregnancy, including increased plasma volume, hemodilution, and increased fetal iron requirements, may reduce maternal hemoglobin concentration, particularly during the second and third trimesters (Benson et al., 2021; O'Toole et al., 2024). To address this condition, iron tablet supplementation has become a standard intervention in antenatal care. However, anemia prevention should not rely solely on pharmacological supplementation, as nutritional intake, dietary behavior, adherence to iron consumption, and access to affordable iron-rich foods also influence maternal hemoglobin concentration (F. Sari et al., 2026).

Spinach (Amaranthus hybridus L.) is a locally available green leafy vegetable that contains non-heme iron, folate, vitamins, minerals, and bioactive nutrients that may support hemoglobin synthesis (Fajrin et al., 2022). In maternal health practice, spinach-based supplementation may provide a complementary nutritional approach to support standard iron therapy (Norhayati et al., 2024; Yastutik & Aminati, 2022). Its availability, affordability, and cultural acceptability make spinach a potentially relevant food-based intervention for community-level anemia prevention, particularly in primary health care settings.

Compared with traditional spinach preparations, such as cooked spinach or spinach juice, spinach extract supplementation combined with iron tablets may offer several practical and methodological advantages (Sari et al., 2024). Traditional preparations are often influenced by variations in portion size, cooking methods, preparation duration, storage conditions, and individual consumption patterns, which may lead to inconsistent nutrient intake (Gelaye, 2023). In contrast, capsule-based supplementation allows a more standardized form of delivery, provides greater dosage consistency, and reduces variability related to food preparation (Sarma et al., 2023). From an adherence perspective, capsules may also be easier for pregnant women to consume because they are portable, simple to administer, and less affected by taste preferences, food aversion, or the time required for meal preparation (Skolmowska et al., 2022). These advantages make spinach extract supplementation combined with iron tablets a potentially relevant adjunct to standard iron tablet supplementation, particularly in primary health care settings where simple, acceptable, and measurable interventions are needed.

Previous spinach-based interventions have generally suggested a potential improvement in hemoglobin concentration among pregnant women with anemia (Qonitun & Suhartatik, 2024). However, the findings remain heterogeneous and should be interpreted with caution. Some studies reported increased hemoglobin concentrations after the administration of spinach juice, cooked spinach, or spinach-based preparations, while other evidence indicates that the effectiveness of green leafy vegetables may depend on preparation method, portion size, duration of intervention, baseline anemia severity, adherence, and the presence of absorption-enhancing nutrients such as vitamin C. (Kusuma, 2023; Soleha et al., 2022; Yastutik & Aminati, 2022). This creates a research gap regarding whether spinach extract supplementation, when combined with standard iron tablets, can provide additional improvement in hemoglobin concentration among pregnant women in primary health care settings.

This study is conceptually grounded in maternal nutrition and iron-deficiency anemia prevention. The relationship between spinach extract supplementation and hemoglobin concentration can be explained through the role of iron and micronutrients in erythropoiesis and hemoglobin synthesis. When combined with standard iron tablets, spinach extract may enhance maternal nutritional support and contribute to improved hemoglobin concentration. Therefore, the independent variable in this study is spinach extract supplementation, while the dependent variable is hemoglobin concentration among pregnant women.

The findings of this study are expected to contribute to nursing, midwifery, and maternal health practice by providing evidence for a locally available complementary nutritional intervention. In clinical practice, the results may support midwives and primary health care providers in strengthening antenatal counseling, monitoring hemoglobin concentration, and promoting adherence to iron supplementation. At the community level, this study may inform maternal anemia prevention strategies using accessible food-based resources.

Methods

Study Design

This study used a quasi-experimental pretest–posttest two-group design to examine the effect of spinach extract supplementation combined with iron tablets on hemoglobin concentration among

pregnant women. The study included an intervention group and a control group. Pregnant women in the intervention group received spinach extract supplementation combined with iron tablets combined with standard iron tablets, whereas those in the control group received standard iron tablets only. Hemoglobin concentration was measured before and after the 14-day intervention period to evaluate within-group changes and between-group differences.

This study was reported in accordance with the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) guideline to improve the transparency and completeness of reporting for nonrandomized intervention studies. Because this study was implemented in a routine primary health care setting, random allocation was not applied. Instead, participants were allocated through a nonrandom assignment process based on existing antenatal care groupings at Baleendah Primary Health Care Center. This design was selected to maintain feasibility within routine antenatal care services while allowing comparison between pregnant women who received spinach extract supplementation combined with iron tablets combined with iron tablets and those who received iron tablets alone

Participants

Participants were recruited from pregnant women attending antenatal care services at Baleendah Primary Health Care Center, Bandung Regency. A non-probability purposive sampling technique was used to select participants who met the eligibility criteria and were considered appropriate for evaluating the effect of spinach extract supplementation on hemoglobin concentration. This sampling strategy was applied to ensure that participants had relevant clinical characteristics, were able to complete the intervention procedures, and could be followed throughout the study period.

The inclusion criteria were pregnant women in the first, second, or third trimester; pregnant women receiving antenatal care at Baleendah Primary Health Care Center; participants who were willing to take part in the study and provided informed consent; and participants who were able to follow the study procedures until completion. The exclusion criteria were pregnant women with pregnancy complications, those who declined participation, and those who were unable to complete the intervention or hemoglobin assessment procedures.

The sample size was determined using a pragmatic feasibility-based approach rather than a formal a priori power analysis. This approach was selected because the study was conducted within a routine antenatal care setting and depended on the number of eligible pregnant women who could be recruited and followed during the study period. A total of 60 participants were included, with 30 pregnant women allocated to the intervention group and 30 pregnant women allocated to the control group. The use of equal group sizes was intended to maintain balance between comparison groups and support the analysis of pretest–posttest hemoglobin changes between the two groups. The feasibility-based sample size also considered the 14-day intervention period, participant availability, and the operational capacity of the primary health care setting.

Instruments

This study involved three types of variables: the independent variable, the dependent variable, and monitoring variables. The independent variable was the type of intervention, consisting of spinach extract supplementation combined with standard iron tablets in the intervention

group and standard iron tablets alone in the control group. The dependent variable was hemoglobin concentration, measured before and after the 14-day intervention period. The monitoring variables included adherence to spinach extract consumption, completion of the intervention period, and follow-up confirmation during the study.

Hemoglobin concentration was measured using the EasyTouch GCHb hemoglobin meter, a portable point-of-care testing device used to assess hemoglobin concentration from capillary blood samples. Hemoglobin values were recorded in grams per deciliter (g/dL), producing ratio-scale data. The device was used without modification and operated according to the manufacturer's instructions. Before data collection, quality assurance procedures were performed by checking device function, battery condition, test strip code compatibility, strip expiration date, storage condition, and cleanliness of the device. The same device type and measurement procedure were used for both pretest and posttest assessments to minimize measurement variability.

Hemoglobin measurements were conducted by trained research personnel in coordination with primary health care providers at Baleendah Primary Health Care Center, Bandung Regency, Indonesia. The personnel involved in hemoglobin assessment received prior orientation regarding capillary blood collection, operation of the EasyTouch GCHb device, infection prevention procedures, participant safety, and standardized recording of results. Each measurement was performed using a new sterile lancet and test strip, and the result displayed on the device was immediately recorded on the observation sheet.

The EasyTouch GCHb device was selected because it provides rapid hemoglobin assessment and is feasible for use in primary health care and field-based settings. Previous studies have reported that digital point-of-care hemoglobin devices are useful for anemia screening and hemoglobin monitoring, although measurement accuracy may vary compared with laboratory-based hematology analyzers. Therefore, standardized procedures were applied during measurement to reduce potential measurement error.

A researcher-developed observation sheet was used to record outcome and monitoring data. The observation sheet was developed based on the study protocol and standard operating procedures for spinach extract administration and hemoglobin assessment. The content of the observation sheet was reviewed by experts in maternal health and research methodology to ensure relevance, clarity, and consistency with the study objectives. Before use in the main study, the observation sheet was pilot tested to assess clarity, feasibility, and consistency of recording.

The observation sheet contained the following items: participant identification code, age, gestational age, group allocation, date of pretest hemoglobin measurement, baseline hemoglobin value, date of posttest hemoglobin measurement, posttest hemoglobin value, daily spinach extract capsule consumption, daily iron tablet consumption, adverse symptoms or complaints, WhatsApp follow-up confirmation, completion of the 14-day intervention, and remarks from the research team. The complete observation sheet is provided as supplementary material.

Adherence to spinach extract supplementation was monitored daily in the intervention group using the observation sheet and WhatsApp follow-up. Adherence was calculated as the number of prescribed spinach extract doses consumed divided by the total number of prescribed doses during the 14-day intervention

period and multiplied by 100. Daily adherence was recorded as "yes" when the participant consumed the prescribed dose according to the study protocol and "no" when the participant missed the dose. Participants were categorized as adherent if they consumed at least 80% of the prescribed supplementation doses during the intervention period. This adherence record was used only as a monitoring variable and was not treated as the primary outcome variable.

Intervention

The intervention was conducted in the antenatal care setting at Baleendah Primary Health Care Center, Bandung Regency, Indonesia. The selection of spinach extract supplementation was based on the biological rationale that spinach-derived micronutrients may support iron metabolism, erythropoiesis, and hemoglobin synthesis. Spinach contains non-heme iron, folate, vitamin C, minerals, and bioactive compounds that may contribute to blood formation. Non-heme iron provides an additional plant-based iron source, folate supports DNA synthesis required for erythrocyte production, and vitamin C may enhance non-heme iron absorption by facilitating the reduction of ferric iron to ferrous iron. Therefore, spinach extract supplementation was used as an adjunct nutritional intervention to support the expected improvement in hemoglobin concentration among pregnant women receiving standard iron tablets.

Both groups received routine antenatal care, including standard iron tablet supplementation, hemoglobin monitoring, and maternal nutrition counseling. The standard iron tablet administered to both groups was an iron–folic acid tablet containing 60 mg elemental iron and 400 µg folic acid per tablet. The tablets were provided through the routine antenatal care program at Baleendah Primary Health Care Center, Bandung Regency, Indonesia, and were administered according to the standard maternal health protocol for anemia prevention during pregnancy. Participants in the control group received standard iron tablets only during the primary observation period, whereas participants in the intervention group received spinach extract supplementation combined with iron tablets in addition to standard iron tablets.

The spinach extract supplementation combined with iron tablets were prepared in collaboration with the Natural Product Pharmaceutical Biology Laboratory at Sekolah Tinggi Farmasi Indonesia. Fresh green spinach leaves (*Amaranthus hybridus L.*) were obtained from organic spinach farmers. During the sorting stage, fresh young green spinach leaves were selected, cleaned, and washed. A total of 5 kg of fresh spinach leaves was dried in an oven at 50°C for 1 hour to obtain dried simplicia. The dried simplicia was then blended into powder.

The extraction process was performed using 70% ethanol as the solvent. A total of 1,000 g of spinach simplicia powder was soaked in 10 L of 70% ethanol for 3 × 24 hours at room temperature with repeated stirring. During maceration, the solvent was replaced to optimize the extraction of remaining active compounds. After 3 × 24 hours, the filtrate was filtered using filter paper and concentrated using a water bath. The macerated extract was further thickened using a water bath and then dried in an oven at 70°C until a thick extract was obtained.

The thick spinach extract was formulated into 1,000 capsules. Capsule filling was performed manually according to the laboratory standard operating procedure by inserting the prepared spinach extract into capsule shells, closing the capsules, and cleaning the capsule surface with tissue before packaging. The

intervention dose was standardized based on the number of capsules administered. Participants in the intervention group consumed two capsules per dose, three times daily, after meals, at 8-hour intervals, for 14 days. Therefore, the total daily dose was six capsules per day. Participants who had difficulty swallowing capsules were allowed to open the capsule and mix the contents with approximately two spoonfuls of water before consumption.

Capsules were packaged in clean, closed containers and labeled according to participant code and administration schedule. The capsules were stored in a clean, dry, closed place protected from direct sunlight. Before distribution, capsule quality assurance was conducted by checking capsule integrity, cleanliness, packaging condition, labeling, and absence of visible physical damage. Standardization of the spinach extract was based on uniform raw material source, controlled drying temperature, fixed solvent concentration, fixed maceration duration, standardized filtration and concentration procedures, consistent capsule preparation, and controlled storage conditions. Active compound quantification, capsule mass uniformity testing, and extraction yield analysis were not performed; therefore, this limitation was acknowledged in the study.

A concise standard operating procedure was developed to ensure reproducibility of the intervention and is provided as supplementary material. The SOP covered raw material selection, washing and drying procedures, powder preparation, maceration, filtration, concentration, capsule preparation, packaging, storage, participant instruction, administration schedule, adherence monitoring, safety monitoring, and documentation.

The intervention was administered and monitored by the research team in coordination with primary health care providers. The research team was responsible for explaining the intervention procedure, distributing capsules, providing written and verbal instructions, monitoring adherence, documenting capsule consumption, conducting WhatsApp follow-up, and recording participant complaints. Primary health care providers supported participant coordination during antenatal care visits, reinforced routine iron tablet counseling, and assisted in monitoring participant safety when needed.

Intervention fidelity was monitored using a structured compliance checklist, daily consumption records, and WhatsApp confirmation. Participants were asked to confirm capsule consumption each day and to report missed doses or complaints. Adherence was calculated as the number of capsules consumed divided by the total number of prescribed capsules during the 14-day intervention period and multiplied by 100. Because the prescribed dose was six capsules per day for 14 days, the total prescribed dose was 84 capsules during the intervention period. Participants were categorized as adherent if they consumed at least 80% of the prescribed capsules. Any missed doses, delayed doses, discontinuation, or complaints were recorded on the monitoring sheet.

Safety monitoring was conducted throughout the 14-day supplementation period. Participants were asked to report adverse events or complaints, including nausea, vomiting, abdominal discomfort, diarrhea, constipation, dizziness, itching, allergic reactions, or other symptoms occurring after capsule consumption. All complaints were recorded by the research team and followed up in coordination with primary health care providers. Participants who experienced severe symptoms were advised to stop supplementation and seek clinical assessment.

Before the intervention, hemoglobin concentration was measured in both groups

as the baseline assessment. After completion of the 14-day intervention period, hemoglobin concentration was reassessed in both groups to evaluate changes before and after supplementation. To uphold the ethical principle of justice, participants in the control group were offered spinach extract supplementation after the posttest assessment had been completed. This delayed supplementation was not included in the primary comparative analysis.

Data Collection

Data were collected at Baleendah Primary Health Care Center, Bandung Regency, Indonesia, from May to June 2025. The data collection process was conducted by the research team in coordination with primary health care providers after obtaining administrative approval and written informed consent from eligible participants. Potential participants were identified from pregnant women attending antenatal care services during the recruitment period. The research team screened participants based on the inclusion and exclusion criteria, explained the study objectives and procedures, obtained written informed consent, and performed baseline hemoglobin measurement. Participants were then allocated into the intervention and control groups using a non-random allocation process. The intervention group received spinach extract supplementation combined with iron tablets combined with standard iron tablets, while the control group received standard iron tablets only during the primary observation period. Follow-up monitoring was conducted for 14 days using daily records and WhatsApp communication. After the intervention period, hemoglobin concentration was reassessed in both groups.

Data collection was performed by the research team with a midwifery and maternal health background. Primary health care providers assisted with participant coordination, routine antenatal care procedures, and safety monitoring. Before data collection, all personnel received standardized briefing on the study protocol, eligibility screening, informed consent, hemoglobin measurement using the EasyTouch device, observation sheet completion, adherence monitoring, adverse event recording, confidentiality, and data verification.

Hemoglobin measurement was performed using a standardized procedure. Before each measurement, the device function, test strip compatibility, expiration date, and storage condition were checked. Capillary blood was collected using a sterile lancet, and the hemoglobin value displayed on the device was immediately recorded on the observation sheet. Formal inter-rater reliability testing was not conducted because hemoglobin concentration was generated by a digital device and recorded as numerical data. Consistency was maintained through standardized procedures, training, device checking, and verification of completed forms.

Data were first recorded on paper-based observation sheets and then entered into Microsoft Excel before analysis using IBM SPSS Statistics version 26. Paper forms were stored securely, and electronic files were password-protected. Quality control included checking completed forms, verifying data entry against original records, and reviewing inconsistent values before analysis. No missing primary outcome data occurred; therefore, all 60 participants were included in the final analysis using a complete-case analysis approach.

Data Analysis

Data were analyzed using IBM SPSS Statistics version 26. Descriptive statistics were used to summarize participant

characteristics and hemoglobin outcomes. Categorical variables were presented as frequencies and percentages, while continuous variables were described using means, standard deviations (SD), minimum values, and maximum values.

The assumption of normality was assessed using the Shapiro–Wilk test due to the sample size of fewer than 50 participants per group. The results showed that hemoglobin data were normally distributed in both groups, with p-values greater than 0.05 for all measurements. In the intervention group, the p-values were 0.432 (pretest) and 0.463 (posttest), while in the control group the p-values were 0.474 (pretest) and 0.126 (posttest), indicating that parametric statistical tests were appropriate.

Homogeneity of variance was assessed using Levene's test prior to conducting the independent-samples t-test for between-group comparisons of hemoglobin change scores. The results of Levene's test were used to determine the assumption of equal variances. For inferential analysis, a paired-samples t-test was used to assess within-group differences in hemoglobin concentration before and after the intervention. An independent-samples t-test was used to compare the mean change in hemoglobin concentration between the intervention and control groups. Statistical significance was set at $p < 0.05$.

To provide a more comprehensive interpretation of the findings, 95% confidence intervals and effect size (Cohen's d) were reported for mean differences. These measures were included to assess both the statistical significance and the magnitude of the intervention effect. No missing primary outcome data were observed because all 60 participants completed both pretest and posttest hemoglobin assessments. Therefore, a complete-case analysis approach was applied.

Ethical Considerations

This study was conducted in accordance with ethical principles for health research involving human participants, including respect for autonomy, beneficence, non-maleficence, justice, confidentiality, and voluntary participation. Ethical clearance was obtained from the Health Research Ethics Committee of Sekolah Tinggi Ilmu Kesehatan Dharma Husada, Bandung, Indonesia, with approval number No.157/KEPK/SDHB/B/V/2025.

Written informed consent was obtained from all participants prior to enrollment. Participants were provided with complete information regarding the study objectives, procedures, potential risks and benefits, confidentiality protection, and their right to withdraw from the study at any time without any consequences to their routine antenatal care services.

To ensure confidentiality and anonymity, each participant was assigned a unique coded identifier. Personal identifying information was separated from research data and was not included in the analytical dataset. Access to research data was restricted only to the principal investigator and authorized research team members. All electronic data were stored in password-protected files, while physical documents were kept in locked storage.

Safety monitoring was conducted throughout the study period to identify any adverse events related to spinach extract supplementation. Participants were actively monitored for possible side effects such as nausea, vomiting, abdominal discomfort, diarrhea, constipation, dizziness, allergic reactions, or other complaints. All adverse events were documented and managed in coordination with primary health care providers. Participants experiencing significant symptoms were advised to discontinue

supplementation and receive appropriate clinical evaluation if necessary.

Results

Table 1. Characteristics of Participants (n=60)

Respondent Characteristics	Intervention Group n = 30	Control Group n = 30	p-value
Maternal Age			
< 20 years	1 (3.3%)	0 (0.0%)	0.601**
20–35 years	27 (90.0%)	28 (93.3%)	
> 35 years	2 (6.7%)	2 (6.7%)	
Gestational Age			
Second trimester	22 (73.3%)	22 (73.3%)	1.000*
Third trimester	8 (26.7%)	8 (26.7%)	
Parity			
Primigravida	9 (30.0%)	9 (30.0%)	1.000*
Multigravida	21 (70.0%)	21 (70.0%)	
Educational Level			
Elementary school	2 (6.7%)	3 (10.0%)	0.958**
Junior high school	6 (20.0%)	6 (20.0%)	
Senior high school	17 (56.7%)	17 (56.7%)	
College/University	5 (16.7%)	4 (13.3%)	
Occupation			
Housewife	21 (70.0%)	21 (70.0%)	1.000**
Employee	6 (20.0%)	6 (20.0%)	
Trader	3 (10.0%)	3 (10.0%)	

*Note. Data are presented as n (%). *) The p-value was obtained using the Chi-square test. **) The p-value was obtained using Fisher's exact test.*

Table 1 illustrates the comparison of respondent characteristics between the intervention and control groups. The majority of respondents in both groups were aged 20–35 years, with 27 respondents (90.0%) in the intervention group and 28 respondents (93.3%) in the control group. Based on gestational age, most respondents were in the second trimester, with 22 respondents (73.3%) in each group. In terms of parity, most respondents were multigravida, with 21 respondents (70.0%) in both the intervention and control groups. Regarding educational level, the majority of respondents had completed senior high school, with 17 respondents (56.7%) in each group. Based on occupation, most respondents were housewives, with 21 respondents (70.0%) in both groups. The statistical analysis showed that all p-values were greater than 0.05, indicating that there were no significant differences in respondent characteristics between the intervention and control groups. Therefore, both groups were considered homogeneous in terms of baseline demographic characteristics.

Table 2. Hemoglobin concentration Before and After Intervention

Variable	Intervention Group	Control Group
Pre-test hemoglobin concentration		
Mean ± SD, g/dL	11.41 ± 0.62	11.44 ± 0.60
Minimum–maximum, g/dL	10.7–12.6	10.4–12.6
Hb < 11 g/dL, n (%)	7 (23.3)	7 (23.3)
Hb ≥ 11 g/dL, n (%)	23 (76.7)	23 (76.7)

Variable	Intervention Group	Control Group
Post-test hemoglobin concentration		
Mean ± SD, g/dL	12.53 ± 0.49	12.20 ± 0.51
Minimum–maximum, g/dL	11.6–13.4	11.4–12.9
Hb < 11 g/dL, n (%)	0 (0.0)	0 (0.0)
Hb ≥ 11 g/dL, n (%)	30 (100.0)	30 (100.0)

Note. Hb= Hemoglobin; SD= Standard Deviation

Table 2 presents the hemoglobin concentration of respondents before and after the intervention in both groups. Before the intervention, the mean hemoglobin concentration in the intervention group was 11.41 ± 0.62 g/dL, while the control group had a mean hemoglobin concentration of 11.44 ± 0.60 g/dL. The minimum and maximum hemoglobin concentrations were 10.7–12.6 g/dL in the intervention group and 10.4–12.6 g/dL in the control group. In both groups, 7 respondents (23.3%) had hemoglobin concentrations below 11 g/dL, while 23 respondents (76.7%) had hemoglobin concentrations of 11 g/dL or higher. After the intervention, the mean hemoglobin concentration increased to 12.53 ± 0.49 g/dL in the intervention group and 12.20 ± 0.51 g/dL in the control group. The minimum and maximum hemoglobin concentrations were 11.6–13.4 g/dL in the intervention group and 11.4–12.9 g/dL in the control group.

Table 3. Comparison of Hemoglobin Concentration Before and After Intervention

Analysis		Intervention Group (n = 30)	Control Group (n = 30)	Mean Difference (95% CI)	t-value (df)	p-value	Cohen's d
Pre-Test	mean ± SD, g/dL	11.41 ± 0.62	11.44 ± 0.61	-0.03 (-0.35 to 0.29)	-0.19 (58)	0.851	
	min–max, g/dL	10.7–12.6	10.4–12.6				
Post-Test	mean ± SD, g/dL	12.53 ± 0.49	12.20 ± 0.51	0.33 (0.07 to 0.59)	2.54 (58)	0.014	
	min–max, g/dL	11.6–13.4	11.4–13.0				
Within-group hemoglobin change	mean ± SD, g/dL	1.12 ± 0.32	—	1.12 (1.00 to 1.24)	19.09 (29)	< 0.001	
	mean ± SD, g/dL	—	0.76 ± 0.33	0.76 (0.64 to 0.88)	12.51 (29)	< 0.001	
Between-group difference in hemoglobin change	mean ± SD, g/dL	1.12 ± 0.32	0.76 ± 0.33	0.36 (0.19 to 0.53)	4.29 (58)	< 0.001	1.11

Note. Note. CI = confidence interval; SD = standard deviation; df = degrees of freedom. Baseline and post-intervention between-group comparisons were analyzed using independent-samples t-test. Within-group hemoglobin changes from pretest to posttest were analyzed using paired-samples t-test. The between-group difference in hemoglobin change was analyzed using independent-samples t-test on change scores. Cohen's d for between-group comparisons refers to standardized mean difference between groups.

Table 3 shows the outcome analysis of hemoglobin concentrations in the intervention and control groups. At pre-test, there was no significant difference in mean hemoglobin concentrations between the intervention group and the control group (11.41 ± 0.62 g/dL vs. 11.44 ± 0.61 g/dL; p = 0.851), indicating that both groups were comparable at baseline. At post-test, the intervention group had a significantly

higher mean hemoglobin concentration than the control group (12.53 ± 0.49 g/dL vs. 12.20 ± 0.51 g/dL; p = 0.014).

Within-group analysis showed that hemoglobin concentrations increased significantly in both groups after treatment, with a mean increase of 1.12 ± 0.32 g/dL in the intervention group and 0.76 ± 0.33 g/dL in the control group ($p < 0.001$ for both). The between-group comparison showed that the increase in hemoglobin was significantly greater in the intervention group than in the control group, with a mean difference of 0.36 g/dL (95% CI: 0.19–0.53; $p < 0.001$). The Cohen's d value of 1.11 indicates a large effect size, suggesting that the intervention was more effective in increasing hemoglobin concentrations.

Discussion

This study showed that spinach extract supplementation combined with standard iron tablets produced a greater improvement in hemoglobin concentration than standard iron tablets alone. Rather than indicating that spinach extract should replace iron supplementation, the findings suggest that spinach extract may function as an adjunct nutritional strategy within routine antenatal care. This interpretation is important because iron tablets remain the standard approach for maternal anemia prevention and management, while food-based supplementation may provide additional nutritional support, improve acceptability, and strengthen counseling on locally available iron-rich foods.

Although the between-group difference in hemoglobin change was statistically significant, the absolute magnitude of the difference should be interpreted carefully. The intervention group showed an additional mean increase of 0.36 g/dL compared with the control group, which indicates a modest absolute improvement despite the large standardized effect size. This distinction is important because statistical significance reflects the probability that the observed difference is unlikely to be due to chance, whereas clinical significance depends on whether the magnitude of improvement is meaningful for patient care, anemia classification, symptom reduction, or maternal and fetal outcomes. In routine antenatal care, an additional increase of 0.36 g/dL may be relevant as a complementary short-term improvement when integrated with standard iron supplementation and nutrition counseling. However, this magnitude should not be interpreted as evidence of substantial or sustained correction of maternal anemia.

Compared with previous trials and reviews of nutritional or food-based interventions for anemia during pregnancy, hemoglobin responses have varied according to baseline anemia severity, intervention duration, formulation, dose consistency, adherence, dietary intake, and the presence of iron absorption enhancers or inhibitors (Rika et al., 2025). The clinical relevance of the finding lies in the possibility of integrating a simple, locally acceptable, and standardized plant-based supplement into existing maternal health services. In primary health care settings, anemia management often depends not only on the availability of iron tablets but also on adherence, dietary behavior, food access, and routine hemoglobin monitoring. The greater improvement observed in the intervention group may therefore reflect the combined contribution of pharmacological iron supplementation and supportive micronutrients from spinach extract. This finding supports the concept that maternal anemia prevention should be addressed through a combined strategy involving supplementation, dietary improvement, counseling, and follow-up monitoring.

Biologically, the potential effect of spinach extract can be explained through

several complementary mechanisms. Spinach contains non-heme iron, which may contribute to hemoglobin synthesis when absorbed adequately (Kusuma, 2023; Natalina & Legawati, 2024). Non-heme iron absorption is influenced by its conversion from ferric iron to the more absorbable ferrous form in the intestinal lumen. Vitamin C contained in green leafy vegetables may support this process by enhancing iron solubility and improving non-heme iron absorption (Hasyimi et al., 2025). In addition, folate plays an important role in DNA synthesis and red blood cell formation, making it relevant for erythropoiesis during pregnancy. Spinach also contains antioxidant compounds, such as vitamins and carotenoids, which may help reduce oxidative stress and support a physiological environment for red blood cell production (Suryati et al., 2022). Therefore, the effect of spinach extract should not be understood only as an additional source of iron, but also as a source of micronutrients that may support iron metabolism and erythropoiesis.

The findings are consistent with several previous studies reporting that spinach-based interventions, such as spinach juice, cooked spinach, or spinach extract, were associated with increased hemoglobin concentration among pregnant women. These studies support the potential role of spinach as a complementary food-based intervention in maternal anemia management (Natalina & Legawati, 2024; E. Sari et al., 2024; Suryati et al., 2022; Umu Qonitun & Suhartatik, 2024). However, the current study differs from many earlier studies because the intervention was administered in capsule form and combined with standard iron tablets. The capsule formulation may offer better standardization than fresh spinach preparations, which can vary in serving size, cooking method, nutrient retention, and participant compliance. This distinction is important because the form of intervention may influence both dose consistency and acceptability.

Nevertheless, findings from food-based and plant-based iron interventions are not always consistent. Previous systematic reviews have shown that dietary interventions for iron-deficiency anemia in pregnant women and women of reproductive age may produce variable effects on hemoglobin and iron status, depending on the type of intervention, dietary composition, duration, and participant characteristics. These discrepancies may be explained partly by the fact that iron from plant-based foods is mainly present as non-heme iron, which has lower and more variable bioavailability than heme iron (Skolmowska et al., 2022). Non-heme iron absorption may be reduced by dietary inhibitors such as phytates, polyphenols, tannins, and calcium, while vitamin C and other dietary enhancers may improve absorption. In the case of spinach, the role of oxalate should be interpreted cautiously, as evidence suggests that oxalic acid itself may not directly reduce non-heme iron absorption, while calcium and polyphenol content may contribute more strongly to lower iron availability from spinach-based meals (Piskin et al., 2022). Therefore, differences in baseline anemia severity, gestational age, intervention formulation, dosage, duration, adherence monitoring, dietary intake, infection or inflammation status, and concurrent use of iron tablets may explain why green leafy vegetable or plant-based interventions do not always produce similar hemoglobin responses across studies.

Compared with studies using spinach juice or cooked spinach, the present study used spinach extract supplementation combined with iron tablets for 14 days as an adjunct to routine iron tablet supplementation. This formulation may reduce variation caused by cooking time,

food preparation, portion size, taste preference, and food aversion during pregnancy. However, the short 14-day intervention period should be considered when interpreting the clinical meaning of the findings. Although hemoglobin concentration increased significantly after the intervention, erythropoiesis and iron repletion are physiological processes that generally require a longer duration to produce stable and sustained clinical effects.

The increase observed in this study may therefore represent an early hematological response rather than complete correction of maternal anemia or restoration of iron stores. Hemoglobin concentration may respond earlier than broader indicators of iron status, but longer follow-up is needed to determine whether the improvement is maintained over time. Therefore, the findings should not be interpreted as evidence of long-term effectiveness of spinach extract supplementation in correcting maternal anemia. Instead, they suggest that spinach extract supplementation combined with iron tablets may contribute to short-term improvement in hemoglobin concentration when used as an adjunct to routine antenatal care. Future studies should apply longer intervention and follow-up periods and include additional biomarkers such as serum ferritin, transferrin saturation, and inflammatory markers to evaluate sustained hematological improvement and iron store recovery.

The findings can be linked to a maternal nutrition and iron-deficiency anemia management framework. Within this framework, hemoglobin concentration is influenced by iron intake, iron absorption, erythropoiesis, physiological hemodilution during pregnancy, dietary practices, and adherence to supplementation. Spinach extract may contribute to this framework by providing additional plant-based micronutrients that support iron metabolism, while iron tablets provide a standardized source of elemental iron (Rika & Others, 2025). The interaction between these components may help explain why the combined intervention showed a greater hemoglobin improvement than iron tablets alone (Suryati et al., 2022). Thus, the study contributes theoretically by supporting the idea that food-based complementary strategies can be integrated into biomedical anemia management rather than positioned as separate or alternative approaches.

Local context is also important in interpreting the findings. In Indonesia, green leafy vegetables such as spinach are familiar, affordable, and culturally acceptable (Norhayati et al., 2024). This may support intervention acceptance because the supplement is derived from a food source that is already known in daily meals. However, Indonesian dietary habits may also affect effectiveness. For example, tea and coffee consumption around meal times or supplement intake may reduce non-heme iron absorption, while low intake of vitamin C-rich foods may limit absorption benefits (Benson et al., 2021). Counseling should therefore address not only the consumption of spinach extract and iron tablets but also the timing of intake, avoidance of dietary inhibitors, and inclusion of iron absorption enhancers.

Primary health care conditions may further influence implementation. In routine antenatal care, midwives and health workers are already involved in iron tablet distribution, hemoglobin screening, and nutrition counseling (Abd Rahman et al., 2022). This creates an opportunity to incorporate spinach extract supplementation as part of a structured counseling and monitoring program. The capsule form may be practical for primary health care because it is portable, easier to distribute, and more consistent than fresh food preparation. However,

implementation would require attention to product standardization, safety monitoring, adherence documentation, cost, and clear guidance on its use as an adjunct to standard iron tablets.

The broader applicability of this intervention may extend to other resource-limited settings where anemia during pregnancy remains common and access to diverse iron-rich foods is limited (Finkelstein et al., 2024). A locally sourced plant-based supplement may be useful in community-based maternal health programs, especially when combined with routine iron supplementation and dietary counseling. However, adaptation to other populations should consider differences in food culture, baseline nutritional status, anemia severity, health system capacity, and availability of quality-controlled extract production. The intervention may also be explored among adolescent girls, women of reproductive age, or postpartum women, although evidence from pregnant women should not be directly generalized without further study.

Overall, this study adds evidence to the growing discussion on food-based complementary approaches in maternal anemia management. Its theoretical contribution lies in showing that spinach extract supplementation combined with iron tablets may serve as a standardized form of local food-based supplementation that supports, rather than replaces, standard iron therapy. The findings also highlight the need to understand anemia management as a combination of biological, behavioral, nutritional, and health system factors. Future research should examine longer intervention periods, more diverse populations, biochemical indicators of iron status, dietary intake patterns, and implementation feasibility in different primary health care contexts.

Implications and limitations

This study contributes to maternal anemia management by providing additional evidence that spinach extract supplementation combined with iron tablets may serve as a standardized food-based complementary nutritional approach alongside routine iron supplementation. The findings support the broader concept that anemia prevention during pregnancy should not rely solely on pharmacological supplementation, but may also incorporate locally available nutritional resources to improve dietary support, intervention acceptability, and integration into primary health care counseling. From a scientific perspective, spinach extract supplementation offers a more standardized form of plant-based intervention than fresh or cooked spinach preparations, thereby supporting the development of evidence-based nutritional strategies in maternal health practice. However, these findings should be interpreted cautiously due to the quasi-experimental design, purposive sampling, single-center setting, relatively short intervention duration, indirect adherence monitoring, and potential residual confounding. Hemoglobin concentration during pregnancy may be influenced by dietary iron intake, vitamin C and calcium intake, tea or coffee consumption, adherence to iron tablets, infection or inflammation, gestational age progression, and physiological hemodilution, which were not comprehensively measured or statistically adjusted for in this study. Therefore, although the intervention group showed a greater increase in hemoglobin concentration, the improvement cannot be attributed solely to spinach extract supplementation with complete certainty. Future studies should use randomized controlled designs with larger and more diverse populations, longer follow-up periods, direct adherence assessment, and

more comprehensive control of dietary and clinical confounders to confirm the effectiveness, feasibility, and broader applicability of spinach extract supplementation in maternal anemia management.

Relevance to Practice

The findings of this study are relevant for healthcare professionals, particularly midwives and primary health care providers, in strengthening antenatal nutrition counseling and maternal anemia prevention strategies. Spinach extract supplementation may be considered as a complementary nutritional approach to support routine iron tablet supplementation, especially in settings where locally available plant-based foods are culturally acceptable and affordable. In practice, this approach could be integrated into antenatal care through structured education on iron-rich foods, appropriate timing of iron intake, avoidance of dietary inhibitors, adherence monitoring, and regular hemoglobin assessment. At the institutional level, the findings may support the development of nutrition counseling protocols or maternal health program guidelines that incorporate feasible food-based interventions while maintaining iron tablets as the standard intervention for anemia prevention and management during pregnancy.

Conclusion

This study highlights the potential role of spinach extract supplementation as a complementary strategy to support maternal anemia prevention when combined with standard iron tablet supplementation. The findings contribute to the growing evidence on food-based nutritional approaches in antenatal care, particularly using locally available plant-based resources. Future randomized studies with larger, more diverse populations and longer follow-up periods are needed to confirm its effectiveness, safety, and broader applicability in maternal health programs.

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CrediT Authorship Contributions Statement

Yeti Hernawati: Conceptualization, Methodology, Data Curation, Writing-Original Draft, Supervision

Haerani Korihah: Validation, Formal Analysis, Resources,

Deris Riandi Setiawan: Wiriting-Review & Editing, Vizualitation

Conflicts of Interest

There is no conflict of interest.

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Supplementary Materials

Supplementary File S1: Research Instrument contains the full questionnaire used for data collection.

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