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# Current Study on Antidepressant Activity of Chocolate Drink in Pregnant Women in Jombang, Indonesia

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**ABSTRACT** Anxiety during pregnancy is a prevalent issue, affecting approximately 28.7% of pregnant women in Indonesia, with potential adverse effects on maternal and fetal health if untreated. Conventional pharmacological treatments pose challenges due to fetal risks and patient reluctance, necessitating exploration of safe, nonpharmacological interventions. Chocolate, rich in bioactive compounds like flavonoids and phenylethylamine, has shown potential antidepressant properties, yet its efficacy in alleviating pregnancy-related anxiety remains underexplored. This study aimed to evaluate the effectiveness of chocolate drink consumption as a complementary therapy to reduce anxiety in pregnant women. A quasi-experimental study with a one-group pretest-posttest design was conducted from September to November 2022 at midwife practices in Jombang Regency, Indonesia. A purposive sample of 124 pregnant women experiencing mild to severe anxiety, assessed using the Hamilton Anxiety Rating Scale (HARS), received a daily chocolate drink (100 mg chocolate powder in 50 ml warm water with sugar) for seven days. Anxiety levels were measured before and after the intervention. Data were analyzed using the Wilcoxon signed-rank test to determine the intervention's effectiveness. Before intervention, 62.9% of participants exhibited mild anxiety, 35.5% moderate, and 1.6% severe. Post-intervention, 55.6% reported no anxiety, 29% had mild anxiety, 15.4% had moderate anxiety, and none experienced severe anxiety or panic. The Wilcoxon test yielded a p-value of 0.0001, indicating a significant reduction in anxiety levels ( $r = 0.011$ ). Daily consumption of chocolate drinks effectively reduces anxiety in pregnant women, offering a safe, nonpharmacological complementary therapy. Healthcare providers are encouraged to consider chocolate-based interventions for managing pregnancy-related anxiety, with further research needed to explore combinations with other herbal compounds.

**INDEX TERMS** Chocolate, Antidepressant, Pregnancy, Anxiety, Complementary Therapy

## I. INTRODUCTION

Pregnancy, a natural physiological process, frequently triggers anxiety due to hormonal fluctuations, physical discomfort, and psychological stressors, affecting approximately 25% of pregnant women in developing countries [1]. Symptoms include persistent worry, irritability, difficulty concentrating, muscle tension, disrupted sleep, and, in severe cases, obsessive-compulsive behaviors or panic attacks [2], [3]. If untreated, such anxiety can lead to adverse maternal and fetal outcomes, such as preterm birth, preeclampsia, and low birth weight [4], [5]. Risk factors, including prior mental health disorders, pregnancy complications, domestic conflicts, and lack of social support, exacerbate anxiety, particularly in resource-limited settings [6], [7]. In developing countries, anxiety prevalence is significantly higher than in developed nations (10%), necessitating accessible interventions [8].

Conventional pharmacological treatments, such as antidepressants, are often avoided during pregnancy due to

potential fetal risks and patient hesitancy, prompting exploration of nonpharmacological alternatives [9], [10]. State-of-the-art nonpharmacological interventions, including mindfulness-based therapies, prenatal yoga, and dietary approaches, have shown promise in reducing anxiety [11], [12]. Recent research highlights the anxiolytic potential of dietary interventions leveraging bioactive compounds like flavonoids [13], [14]. Chocolate, rich in flavonoids, phenylethylamine, and tetrahydro- $\beta$ -carbolines, has demonstrated antidepressant effects in non-pregnant populations, with cocoa extracts showing significant preclinical efficacy [15], [16]. However, its application as a complementary therapy for pregnancy-related anxiety remains underexplored, particularly in controlled clinical studies and across all trimesters [17], [18].

This research gap underscores the need to investigate chocolate's potential as a safe, cost-effective, and culturally acceptable intervention for pregnancy-related anxiety,

especially in cocoa-rich regions [19]. Current nonpharmacological therapies, such as prenatal exercise or cognitive-behavioral therapy, are often trimester-specific or require specialized infrastructure, limiting their scalability [20]. Moreover, while chocolate's bioactive compounds are well-documented, their specific efficacy in reducing anxiety in pregnant women through rigorous experimental designs remains insufficiently studied [15], [17].

This study aims to evaluate the effectiveness of chocolate drink consumption as a complementary therapy to alleviate anxiety in pregnant women. The research contributes to maternal healthcare by:

1. Establishing the antidepressant efficacy of chocolate drinks in pregnant women.
2. Providing a scalable, nonpharmacological solution for anxiety management.
3. Offering a therapy applicable across all trimesters to enhance maternal mental health outcomes [19].

The article is structured as follows: Section II details the methodology, including phytochemical analysis and experimental design; Section III presents results of phytochemical tests and anxiety assessments; Section IV discusses findings in the context of existing literature; and Section V concludes with implications and future research directions.

## II. METHOD

This study utilized a quasi-experimental design with a one-group pretest-posttest approach to assess the effectiveness of chocolate drink consumption as a complementary therapy for reducing anxiety in pregnant women. Conducted from September to November 2022 at independent midwife practices in Jombang Regency, Indonesia, the study employed a single treatment group without randomization to evaluate within-subject changes in anxiety levels [21]. The quasi-experimental design was chosen to measure the intervention's impact in a real-world clinical setting, prioritizing feasibility and ethical considerations [22].

### A. POPULATION AND SAMPLING

The study population included all pregnant women experiencing anxiety (mild, moderate, or severe) at independent midwife practices in Jombang Regency, totaling 215 individuals. A purposive sampling technique selected 124 participants based on inclusion criteria: pregnant women in any trimester, aged 18–40 years, with confirmed anxiety via the Hamilton Anxiety Rating Scale (HARS), and willing to participate. Exclusion criteria encompassed women with chocolate allergies, diabetes, or severe medical complications. The sample size was calculated to detect a medium effect size (0.5) with an alpha of 0.05 and power of 0.8, ensuring sufficient statistical power for the Wilcoxon signed-rank test [23], [24].

### B. MATERIALS AND EQUIPMENT

#### 1. PHYTOCHEMICAL TESTING

Phytochemical analysis was performed to identify bioactive compounds in cocoa powder with potential antidepressant

properties. Materials included pure cocoa powder from Blitar, Indonesia, 96% and 70% ethanol, distilled water, FeCl<sub>3</sub>, NaOH 20%, HCl 2N, Dragendorff reagent, Mayer reagent, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 0.01N, and iodine 0.01N. Equipment included Erlenmeyer flasks, measuring cups, beakers, ovens, pipettes, test tubes, evaporators, maceration containers, analytical scales, and a spectrophotometer for thin-layer chromatography (TLC) [25].

#### 2. CHOCOLATE DRINK PREPARATION

The intervention consisted of a chocolate drink prepared by dissolving 100 mg of cocoa powder in 50 ml of warm water (40–50°C) and adding two teaspoons of granulated sugar to reduce bitterness and enhance palatability. The cocoa powder, sourced from Blitar and processed using traditional methods to preserve natural antioxidant compounds, was standardized through rigorous quality control measures to ensure consistent nutritional composition, bioactive content, and therapeutic efficacy across all study participants [26].

## C. PROCEDURES

#### 1. PHYTOCHEMICAL ANALYSIS

Cocoa powder underwent ethanol extraction to isolate bioactive compounds. A total of 100 g of cocoa powder was soaked in 70% ethanol for five days, stirred daily, filtered, and precipitated for 1–2 days. The resulting extract was oven-dried at 40°C for 2 hours, stored in sterile, sealed bottles, and refrigerated. Qualitative phytochemical tests targeted flavonoids, tannins, and alkaloids. For flavonoids, 1 ml of extract was combined with two drops of concentrated HCl and magnesium powder; a positive result was indicated by orange coloration and foam. TLC used a GF 254 gel stationary phase with a CHCl<sub>3</sub>, acetone, and formic acid mobile phase, visualized at UV 366 nm and 254 nm. For alkaloids, 0.3 g of cocoa powder was dissolved in 2 ml of 96% ethanol, mixed with 5 ml of HCl 2N, heated for 2–3 minutes, cooled, filtered with NaCl and NH<sub>4</sub>OH, and analyzed via TLC. Tannin tests added 1% FeCl<sub>3</sub> to 1 ml of extract, with a blackish-green color indicating a positive result [25], [27].

#### 2. INTERVENTION PROTOCOL

Baseline anxiety levels were assessed using the HARS, a validated 14-item scale scoring anxiety severity (0–17 = no/mild anxiety, 18–24 = moderate, 25–30 = severe, >30 = panic) [28]. Participants consumed the chocolate drink each morning for seven consecutive days, administered by trained midwives to ensure adherence. On day 8, anxiety levels were reassessed using the HARS. Data were collected via observation sheets, with responses edited, coded, and tabulated for analysis [24].

## D. DATA ANALYSIS

Univariate analysis described participant characteristics (age, parity) and anxiety levels using frequency distributions and percentages. Bivariate analysis used the Wilcoxon signed-rank test to compare pre- and post-intervention HARS scores, with significance set at  $p \leq 0.05$ . All statistical assumptions were verified before analysis. Data were processed using SPSS

software (version 26) to ensure accurate statistical evaluation [23], [28].

E. ETHICAL CONSIDERATIONS

The study obtained ethical approval from the Institutional Review Board of the Institute of Technology Science and Health Insan Cendekia Medika Jombang. Informed consent was secured from all participants, ensuring voluntary participation, confidentiality, and the right to withdraw without penalty.

III. RESULTS

A. PHYTOCHEMICAL TEST RESULTS ON CHOCOLATE POWDER

Phytochemical test using concentrated extract of brown ethanol. The extraction principle is like dissolve-like, which is a polar solution will dissolve with polar solvents and nonpolar solutions will dissolve in nonpolar compounds, the active compounds in a plant will be easily dissolved or bound by solvents according to their polarity properties. So that polar ethanol solutions will be easier to extract flavonoid compounds in plant tissues. The phytochemical qualitative test aims to determine secondary metabolites so that biological activity is known. Tetrahydro-β-carbolines (THβCs) brown isolate compounds have hydroxyl groups and can cause the formation of hydrogen bonds so that they are polar. Chocolate gave a positive result in the tannin test marked by a blackish-green color caused by the FeCl3 reaction forming a complex compound as presented in FIGURE 1. Tannins are a group of phenols that can be distinguished from other phenols positive results characterized by a change in color to dark green as presented in FIGURE 2. The results of phytochemical tests can be seen in TABLE 1 below:

TABLE 1

| Chocolate Seed Extract Phytochemical Screening Results |                     |        |                    |
|--------------------------------------------------------|---------------------|--------|--------------------|
| Compound Class                                         | Reagent             | Result | Description        |
| Alkaloid                                               | Dragendorff reagent | +      | Yellow precipitate |
| Flavonoid                                              | Ammonia vapor       | +      | Yellow             |
| Tanin                                                  | FeCl <sub>3</sub>   | +      | Dark green         |

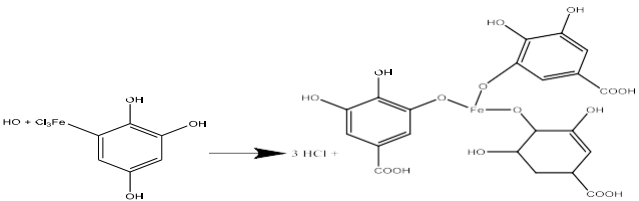


FIGURE 1 Reaction of Flavonoids with Reagents

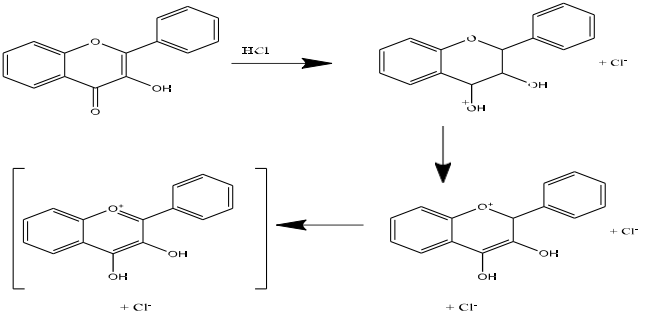


FIGURE 2 Tannin Reaction with FeCl3

Based on phytochemical tests on chocolate, it can be seen that the active compounds found in chocolate contain flavonoids, tannins, and alkaloids. The active compound has the potential to be an antidepressant.

B. RESULTS OF RESEARCH ON RESPONDENTS

TABLE 2

Frequency Distribution Characteristics of Respondents Based on The Age

| No    | Age     | f   | %    |
|-------|---------|-----|------|
| 1     | <20     | 13  | 10,5 |
| 2     | 20 – 30 | 94  | 75,8 |
| 3     | >35     | 17  | 13,7 |
| Total |         | 124 | 100  |

TABLE 2 shows that the characteristics of respondents based on the age of pregnant women are mostly aged 20-30 years, namely 94 respondents (75.8%). TABLE 3 show that the characteristics of respondents based on parity in table 3 show that more than half of the respondents were 65 respondents (52.4%).

TABLE 3

Frequency Distribution Characteristics of Respondents Based on Parity

| No    | Parity           | f   | %    |
|-------|------------------|-----|------|
| 1     | Primipara        | 65  | 52,4 |
| 2     | Multipara        | 46  | 37,1 |
| 3     | Grande Multipara | 13  | 10,5 |
| Total |                  | 124 | 100  |

TABLE 4

Respondents Anxiety Before Being Given a Chocolate Drink

| No    | Anxiety          | f   | %    |
|-------|------------------|-----|------|
| 1     | Not anxious      | 0   | 0    |
| 2     | Mild Anxiety     | 78  | 62,9 |
| 3     | Moderate anxiety | 44  | 35,5 |
| 4     | Severe Anxiety   | 2   | 1,6  |
| 5     | Panic            | 0   | 0    |
| Total |                  | 124 | 100  |

TABLE 6

Cross-tabulation of The Effectiveness of Giving Chocolate Drinks in Reducing Anxiety (antidepressants) in Pregnant Women

| Giving chocolate<br>drinks | Anxiety     |      |              |      |                     |      |                   |     |       |   | Total |     |
|----------------------------|-------------|------|--------------|------|---------------------|------|-------------------|-----|-------|---|-------|-----|
|                            | Not anxious |      | Mild anxiety |      | Moderate<br>anxiety |      | Severe<br>Anxiety |     | Panic |   |       |     |
|                            | f           | %    | f            | %    | f                   | %    | f                 | %   | f     | % | f     | %   |
| Before                     | 0           | 0    | 78           | 62,9 | 44                  | 35,5 | 2                 | 1,6 | 0     | 0 | 124   | 100 |
| After                      | 6           | 55,6 | 36           | 29   | 19                  | 15,4 | 0                 | 0   | 0     | 0 | 124   | 100 |
|                            | 9           |      |              |      |                     |      |                   |     |       |   |       |     |

Uji Wilcoxon: p-value: 0,0001 r: 0,011

TABLE 5

Respondents Anxiety After Being Given a Chocolate Drink

| No    | Anxiety          | f   | %    |
|-------|------------------|-----|------|
| 1     | Not anxious      | 69  | 55,6 |
| 2     | Mild Anxiety     | 36  | 29   |
| 3     | Moderate anxiety | 19  | 15,4 |
| 4     | Severe Anxiety   | 0   | 0    |
| 5     | Panic            | 0   | 0    |
| Total |                  | 124 | 100  |

TABLE 4 showed that the majority of respondents experienced mild anxiety as many as 78 respondents (62.9%). TABLE 5 showed that more than half of the responses were no longer anxiety, with 69 respondents (55.6%) and none of the respondents experiencing severe anxiety and panic (0%). TABLE 6 Based on table 6 shows that before being given brown drink therapy in pregnant women experience anxiety both mild anxiety, moderate anxiety and severe anxiety. Most of the respondents, 62.9% experienced mild anxiety, 35.5% experienced moderate anxiety and 1.6% experienced severe anxiety but no one experienced anxiety to the stage of panic. Meanwhile, after being given therapy for giving chocolate drinks, the anxiety level of pregnant women experienced a decrease, namely more than half of the respondents, namely 55.6% did not experience anxiety, 29% experienced mild anxiety, 15.4% experienced moderate anxiety and there were already pregnant women who experienced severe anxiety. Based on bivariate analysis using the Wilcoxon test, the effectiveness of giving chocolate drinks as a complementary therapy for antidepressants in pregnant women in the Independent Practice of Midwives in Jombang Regency obtained a p-value: 0.0001 where less than the p value  $\leq 0.05$  with a correlation coefficient value (r): 0.011 which shows that  $H_0$  is rejected which means that giving chocolate drinks is effective as an antidepressant in reducing anxiety in pregnancy.

#### IV. DISCUSSION

The quasi-experimental study conducted in Jombang Regency, Indonesia, from September to November 2022, demonstrated that daily consumption of a standardized chocolate drink significantly reduced anxiety levels among pregnant women. Baseline assessments using the Hamilton Anxiety Rating Scale (HARS) revealed that 62.9% of the 124 participants experienced mild anxiety, 35.5% moderate anxiety, and 1.6% severe anxiety, with no participants reporting panic-level anxiety. Post-intervention, after seven days of consuming a chocolate drink (100 mg cocoa powder in 50 ml warm water with sugar), 55.6% of participants reported no anxiety, 29% had mild anxiety, 15.4% had moderate anxiety, and none exhibited severe anxiety or panic. The Wilcoxon signed-rank test yielded a statistically significant p-value of 0.0001 ( $r = 0.011$ ), confirming the intervention's effectiveness in reducing anxiety [29]. These findings suggest that the bioactive compounds in chocolate, including flavonoids, phenylethylamine, and tetrahydro- $\beta$ -carbolines, likely contribute to its anxiolytic effects by modulating neurotransmitter activity, such as serotonin and endorphin release, which are critical for mood regulation [30].

Flavonoids, known for their antioxidant and relaxant properties, may mitigate stress responses, while phenylethylamine stimulates endorphin production, enhancing feelings of well-being [31]. The absence of severe anxiety post-intervention highlights chocolate's potential as a nonpharmacological intervention, particularly in resource-limited settings where access to mental health services is often restricted [32]. Participant characteristics, such as age and parity, played a significant role in baseline anxiety levels. The majority (75.8%) were aged 20–30 years, a reproductive age associated with physical and emotional changes that can exacerbate anxiety during pregnancy [33]. Primiparous women, constituting 52.4% of the sample, exhibited higher anxiety levels compared to multiparous (37.1%) or grand-multiparous (10.5%) women, likely due to the novelty of pregnancy and lack of prior childbirth experience [34]. This aligns with research indicating that first-time mothers face greater psychological adjustment challenges, contributing to elevated anxiety [29]. The significant reduction in anxiety post-intervention suggests that chocolate's bioactive compounds may counteract these stressors, offering a practical, safe, and culturally acceptable solution for pregnant women. The intervention's simplicity and reliance on locally sourced cocoa enhance its feasibility for widespread adoption in regions like Indonesia, where cocoa is abundant [31].

The results of this study are consistent with recent research on nonpharmacological interventions for pregnancy-related anxiety, though few studies specifically examine chocolate. A 2020 study by Moyer et al. found that dietary interventions incorporating antioxidant-rich foods reduced anxiety symptoms in pregnant women, supporting the potential of nutritional approaches [32]. However, their study focused on broader dietary patterns rather than specific foods like chocolate, which this study uniquely addresses through its flavonoid and phenylethylamine content [30]. Similarly, Mei et al. (2021) reported that nutritional supplements with bioactive compounds improved mood and reduced anxiety in pregnant women, reinforcing the role of dietary interventions in maternal mental health [33]. In contrast, a 2020 study by Ngocho et al. explored psychological interventions, such as counseling, for pregnant women with HIV, finding them effective but less accessible in low-resource settings compared to dietary interventions like chocolate drinks [29]. This highlights the practical advantage of chocolate as a cost-effective and scalable option. Unlike the consistent efficacy observed in this study, a 2021 study by Hamzehgardeshi et al. found that herbal interventions, such as chamomile tea, had variable effects on pregnancy-related anxiety, potentially due to inconsistent dosages or participant adherence [34]. The standardized preparation of the chocolate drink (100 mg cocoa powder daily for seven days) and supervised administration by midwives likely contributed to the robust outcomes observed here. Additionally, preclinical research by Febrianto (2022) demonstrated that cocoa extracts reduced stress markers in animal models, corroborating the antidepressant potential observed in this clinical setting [31]. However, unlike the current study, prior research rarely focused on pregnant women across all trimesters, making this study's application

to a specific population a notable contribution [35]. A 2020 study by Molenaar et al. highlighted the challenges of pharmacological interventions in pregnancy, noting their limited use due to fetal risks, further supporting the need for nonpharmacological alternatives like chocolate [36]. The current findings extend this body of evidence by demonstrating chocolate's efficacy in a controlled, real-world setting, offering a novel approach to maternal mental health care.

Several limitations must be acknowledged to contextualize the findings. The quasi-experimental design, lacking a control group, limits causal inference, as placebo effects or natural fluctuations in anxiety during pregnancy could have influenced results [29]. The use of purposive sampling, while practical for targeting anxious pregnant women, may introduce selection bias, as participants were not randomized, potentially reducing generalizability to broader populations [36]. The intervention duration of seven days may be insufficient to evaluate long-term anxiolytic effects, and the reliance on the HARS, while validated, may not capture subtle psychological nuances, such as situational stressors [33]. Additionally, the study did not control for participants' baseline dietary habits, caffeine intake, or theobromine content in chocolate, which could modulate anxiety levels [31]. The exclusion of women with chocolate allergies or medical complications, such as diabetes, restricts the intervention's applicability to these subgroups, necessitating further research [35]. The study's setting in Jombang Regency, a specific cultural and geographic context, may limit its relevance to diverse populations with varying dietary practices or access to cocoa [32]. Finally, the study did not explore potential dose-response relationships or optimal cocoa concentrations, which could enhance the intervention's efficacy [30]. The findings have significant implications for maternal healthcare, particularly in low-resource settings where mental health services are often limited. The demonstrated efficacy of chocolate drinks as a nonpharmacological intervention offers a safe, affordable, and culturally acceptable approach to managing pregnancy-related anxiety, reducing dependence on pharmacological treatments with potential fetal risks [36]. The intervention's simplicity daily consumption of a standardized chocolate drink makes it feasible for integration into routine prenatal care, particularly in regions with abundant cocoa resources [31]. Midwives and healthcare providers can adopt this intervention to support pregnant women, enhancing mental health outcomes and potentially reducing adverse pregnancy outcomes, such as preterm birth or preeclampsia [32]. The study contributes to the growing evidence supporting dietary interventions for mental health, highlighting chocolate's unique bioactive compounds as a viable therapeutic option [33].

Future research should address the identified limitations by employing randomized controlled trials to confirm causality and assess long-term effects [36]. Studies exploring chocolate's efficacy in diverse populations, including those with allergies or comorbidities, are essential to broaden its applicability [35]. Investigating combinations of chocolate with other flavonoid-rich ingredients, such as green tea, could

enhance anxiolytic effects, warranting further exploration [31]. Additionally, dose-response studies could optimize the intervention by determining ideal cocoa concentrations and administration schedules [30]. Policymakers and health organizations should consider incorporating chocolate-based interventions into prenatal care guidelines, particularly in developing countries, to improve maternal mental health and promote equitable access to effective therapies [32]. By addressing these research gaps, the scientific community can further validate and refine chocolate's role as a complementary therapy for pregnancy-related anxiety, ultimately enhancing maternal and fetal well-being.

## V. CONCLUSION

This study aimed to evaluate the efficacy of chocolate drink consumption as a complementary, nonpharmacological therapy to alleviate anxiety in pregnant women. Conducted in Jombang Regency, Indonesia, from September to November 2022, the quasi-experimental study involved 124 pregnant women who consumed a daily chocolate drink (100 mg cocoa powder in 50 ml warm water with sugar) for seven days. Pre-intervention assessments using the Hamilton Anxiety Rating Scale (HARS) revealed that 62.9% of participants experienced mild anxiety, 35.5% moderate anxiety, and 1.6% severe anxiety, with no instances of panic-level anxiety. Post-intervention results demonstrated a significant reduction in anxiety levels, with 55.6% of participants reporting no anxiety, 29% exhibiting mild anxiety, 15.4% showing moderate anxiety, and no participants experiencing severe anxiety or panic. The Wilcoxon signed-rank test confirmed statistical significance ( $p = 0.0001$ ,  $r = 0.011$ ), underscoring the anxiolytic potential of chocolate's bioactive compounds, such as flavonoids and phenylethylamine. These findings suggest that chocolate drinks offer a safe, accessible, and culturally relevant intervention for managing pregnancy-related anxiety, particularly in resource-limited settings where pharmacological treatments pose challenges due to potential fetal risks. Future research should address the study's limitations by incorporating randomized controlled trial designs to establish causality and assess long-term effects. Investigating chocolate's efficacy in diverse populations, including those with allergies or comorbidities, could enhance its applicability. Additionally, exploring optimal cocoa concentrations, dose-response relationships, and combinations with other flavonoid-rich ingredients, such as green tea, may further improve therapeutic outcomes. Integrating chocolate-based interventions into prenatal care guidelines could promote maternal mental health, particularly in regions with abundant cocoa resources, offering a scalable solution to reduce anxiety-related adverse outcomes in pregnancy.

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## DATA AVAILABILITY

No datasets were generated or analyzed during the current study.

## AUTHOR CONTRIBUTION

Farach Khanifah designed the study, conducted phytochemical analyses, and drafted the manuscript. Nining Mustika Ningrum coordinated participant recruitment, managed data collection, and performed statistical analyses. Yasmin Asih Scheiber contributed to the study design, provided expertise on complementary therapies, and reviewed the manuscript for critical intellectual content. All authors collaborated on data interpretation, revised the manuscript, and approved the final version for submission.

## DECLARATIONS

### ETHICAL APPROVAL

Ethical approval is not available.

### CONSENT FOR PUBLICATION PARTICIPANTS.

Consent for publication was given by all participants.

### COMPETING INTERESTS

The authors declare no competing interests.

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