IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Effect of Clitoria ternatea Vaginal Wash on Restoration of Vaginal Lactobacillus-Dominant Microbiome and Reduction of Inflammatory Markers

Dr. DHIVYA C M[1], Dr. ANUSHA R[2], Dr. AMBILI R S[3], Dr. SHYAMALA DEVI P[4]

DEPARTMENT OF NATUROPATHY

SREE RAMAKRISHNA MEDICAL COLLEGE OF NATUROPATHY AND YOGIC SCIENCES, KULASEKHARAM, KANYAKUMARI

BACKGROUND: Recurrent vaginitis is a prevalent gynecological condition associated with disruption of the vaginal microbiome and elevated pro-inflammatory cytokine activity. Conventional pharmacological management, while effective in the short term, is often associated with recurrence and antimicrobial resistance. *Clitoria ternatea* (butterfly pea) is a medicinal plant with documented anti-inflammatory, antimicrobial, and antioxidant properties, yet its clinical application as a topical vaginal wash has not been systematically evaluated.

OBJECTIVE: This study aimed to evaluate the effect of *Clitoria ternatea* vaginal wash on inflammatory markers — specifically interleukin-6 (IL-6) and transforming growth factor-beta (TGF-β) — and on clinical symptom scores in female participants with recurrent vaginitis over a 30-day intervention period.

METHODS: A single-group pre/post pilot study was conducted with ten (n = 10) female participants diagnosed with recurrent vaginitis. Participants applied *Clitoria ternatea* vaginal wash daily over 30 days. Outcome measures — IL-6 (pg/mL), TGF-β (pg/mL), and composite clinical symptom scores — were assessed at Baseline (Day 0), Mid-intervention (Day 14), and End of intervention (Day 30). Statistical analysis employed paired samples t-tests and Wilcoxon Signed-Rank Tests, with significance set at $\alpha = 0.05$.

RESULTS: Significant reductions were observed in all outcome measures from baseline to end of intervention. IL-6 levels declined from 19.99 ± 2.9 pg/mL to 10.55 ± 1.6 pg/mL (47.3% reduction; $t = 22.275$, $p < 0.001$). TGF-β levels decreased from 331.16 ± 30.8 pg/mL to 188.53 ± 17.9 pg/mL (43.1% reduction; $t = 34.273$, $p < 0.001$). Composite clinical symptom scores improved from 11.1 ± 2.2 at baseline to 1.5 ± 1.6 at end of intervention ($t = 36.000$, $p < 0.001$). All findings were corroborated by non-parametric Wilcoxon Signed-Rank Tests ($p = 0.002$ for all comparisons).

CONCLUSION: *Clitoria ternatea* vaginal wash demonstrated significant anti-inflammatory effects and symptomatic relief in women with recurrent vaginitis within a 30-day period. These findings support the biological plausibility of this botanical intervention and provide a foundation for a future randomized controlled trial with a larger sample size and control group.

Keywords: Clitoria ternatea, vaginal wash, vaginitis, interleukin-6, TGF-β, inflammatory markers, clinical symptom score, pilot study, botanical intervention, vaginal microbiome

INTRODUCTION

1.1 Background of the Study

Vaginal health is an essential component of women's overall reproductive and gynecological well-being. The healthy vaginal environment is characterized by a Lactobacillus-dominant microbiome, which maintains an acidic pH and protects against pathogenic colonization through the production of lactic acid, hydrogen peroxide, and bacteriocins. Disruption of this delicate microbial balance — a condition broadly referred to as vaginal dysbiosis — predisposes women to a spectrum of inflammatory conditions, including bacterial vaginosis (BV), vulvovaginal candidiasis, and trichomoniasis, collectively encompassing recurrent vaginitis.[14,20]

Recurrent vaginitis is one of the most prevalent gynecological complaints worldwide, affecting women across all age groups and socioeconomic backgrounds. It is characterized by persistent or repeated episodes of vaginal inflammation, with symptoms including abnormal discharge, malodor, itching, burning, and irritation. Beyond the immediate discomfort, recurrent vaginitis is associated with a range of adverse health outcomes, including increased susceptibility to sexually transmitted infections (STIs) such as human immunodeficiency virus (HIV) and human papillomavirus (HPV), pelvic inflammatory disease, and adverse obstetric outcomes including preterm birth. The psychological impact of recurrent symptoms — including anxiety, reduced self-esteem, and impaired sexual health — further underscores the significant burden this condition places on affected individuals.[14,16]

At the pathophysiological level, recurrent vaginitis is mediated in large part by dysregulated mucosal immune responses. Pro-inflammatory cytokines, particularly interleukin-6 (IL-6) and transforming growth factor-beta (TGF-β), are consistently elevated in women with active vaginal inflammation and have been proposed as both biomarkers of disease activity and mediators of mucosal immune dysregulation. IL-6 is a pleiotropic cytokine that promotes the recruitment of immune effector cells and drives the acute phase inflammatory response, while TGF-β plays a complex immunomodulatory role, with paradoxical elevation observed in chronic inflammatory states. Normalization of these cytokine profiles is therefore a clinically meaningful therapeutic target in the management of recurrent vaginitis.[11,12,13]

1.2 Limitations of Conventional Management

The current standard of care for recurrent vaginitis relies predominantly on pharmacological interventions, including antibiotics (primarily metronidazole and clindamycin for BV), antifungal agents (for candidiasis), and antiprotozoal medications (for trichomoniasis). While these treatments are effective in clearing acute episodes, they are associated with high rates of recurrence — with up to 50–70% of women experiencing relapse within three to twelve months of treatment. Repeated antibiotic exposure contributes to antimicrobial resistance, further disruption of the vaginal microbiome, and increased susceptibility to secondary infections, perpetuating a cycle of recurrence and retreatment.[20][14,20]

These limitations have generated considerable interest in alternative and complementary therapeutic strategies that can address the underlying inflammatory and microbiological imbalances associated with recurrent vaginitis, rather than merely treating acute symptoms. Botanical or plant-derived interventions, with their diverse bioactive phytochemical profiles, represent a promising avenue for such approaches, particularly in topical formulations that allow for targeted delivery to the vaginal mucosa.

1.3 Clitoria ternatea as a Therapeutic Candidate

Clitoria ternatea L. (Fabaceae), commonly known as butterfly pea or blue pea, is a perennial herbaceous legume native to tropical Asia that has been extensively utilized in traditional Ayurvedic and Southeast Asian medicine for centuries. The plant's vivid blue flowers are rich in a diverse array of bioactive phytochemicals, including polyacylated anthocyanins (known as ternatins), flavonoids (particularly quercetin and kaempferol glycosides), tannins, saponins, alkaloids, and phenolic acids. These compounds collectively confer a broad spectrum of pharmacological activities, including potent antioxidant, anti-inflammatory, antimicrobial, antifungal, and immunomodulatory properties.[2,7]

Of particular relevance to the present study, *Clitoria ternatea* extracts have been demonstrated to inhibit the nuclear factor kappa-B (NF-κB) signaling pathway — a master regulator of pro-inflammatory cytokine transcription — through the action of ternatin anthocyanins and flavonol fractions. This mechanistic activity is associated with downstream suppression of IL-6, tumor necrosis factor-alpha (TNF-α), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) expression.[3,5,6] Additionally, the antimicrobial properties of *C. ternatea* against a range of vaginal pathogens, including *Gardnerella vaginalis* and *Candida albicans*, suggest potential utility in restoring microbial balance in the vaginal environment.[5,9]

Despite this promising pharmacological profile, the clinical application of *Clitoria ternatea* as a topical vaginal wash in women with recurrent vaginitis has not been systematically investigated. The formulation of *C. ternatea* as a vaginal wash offers the advantage of direct mucosal contact, potentially maximizing local bioavailability of anti-inflammatory phytochemicals while minimizing systemic absorption. This represents a novel and clinically relevant application of this well-established botanical agent.

1.4 Statement of the Problem

Recurrent vaginitis remains a persistent and inadequately managed clinical problem, with high rates of treatment failure and relapse under conventional pharmacological regimens. The elevated pro-inflammatory cytokine milieu — particularly IL-6 and TGF-β — associated with this condition reflects an underlying state of chronic mucosal immune dysregulation that is not adequately addressed by antimicrobial therapy alone. Despite the well-documented anti-inflammatory and antimicrobial properties of *Clitoria ternatea*, no clinical pilot study has evaluated its efficacy as a topical vaginal wash in reducing inflammatory markers and improving symptom outcomes in women with recurrent vaginitis. This gap in the evidence base represents the primary motivation for the present study.

1.5 Objectives of the Study

1.5.1 General Objective

To evaluate the effect of *Clitoria ternatea* vaginal wash on inflammatory markers (IL-6 and TGF-β) and clinical symptom scores in female participants with recurrent vaginitis over a 30-day intervention period.

1.5.2 Specific Objectives

1. To measure changes in IL-6 levels at baseline, Day 14, and Day 30 following daily application of *Clitoria ternatea* vaginal wash.
2. To measure changes in TGF-β levels at baseline, Day 14, and Day 30 following daily application of *Clitoria ternatea* vaginal wash.
3. To assess changes in composite clinical symptom scores (discharge, odor, itching, burning, and irritation) at baseline, mid-intervention, and end of intervention.
4. To determine the statistical significance of observed changes using paired t-tests and Wilcoxon Signed-Rank Tests.
5. To provide preliminary evidence to support the design of a future randomized controlled trial evaluating *Clitoria ternatea* vaginal wash as a complementary intervention for recurrent vaginitis.

1.6 Significance of the Study

This study makes an original contribution to the emerging body of evidence on botanical interventions for vaginal inflammatory conditions. By providing the first pilot clinical data on the effects of *Clitoria ternatea* vaginal wash on objective inflammatory biomarkers and subjective symptom outcomes, it addresses a significant gap in the literature and offers a foundation for future evidence-based development of this intervention. The findings are of direct relevance to gynecological nursing practice, women's health research, and the broader field of integrative medicine, and may ultimately contribute to improved management options for the large population of women affected by recurrent vaginitis.

1.7 Scope and Delimitation

This study was delimited to female participants with a clinical diagnosis of recurrent vaginitis recruited through a single site. The intervention was restricted to the topical application of Clitoria ternatea vaginal wash over a 30-day period, and outcomes were assessed at three designated time points. The study did not include microbiome profiling, hormonal assessment, or long-term follow-up beyond the intervention period. As a single-group pilot study without a control arm, causal inferences are limited, and findings are intended to be exploratory and hypothesis-generating rather than confirmatory.

1.8 Definition of Terms

Bacterial Vaginosis (BV): A clinical syndrome resulting from the replacement of the normal Lactobacillus-dominant vaginal flora with a polymicrobial anaerobic bacterial community, characterized by elevated vaginal pH, homogeneous discharge, and positive whiff test.

Clitoria ternatea: A tropical leguminous plant of the family Fabaceae, whose flowers contain bioactive phytochemicals with documented anti-inflammatory, antioxidant, and antimicrobial properties.

Interleukin-6 (IL-6): A pleiotropic pro-inflammatory cytokine produced by immune and non-immune cells, involved in the regulation of acute phase inflammatory responses and mucosal immunity.

Transforming Growth Factor-β (TGF-β): An immunoregulatory cytokine with complex roles in inflammation, immune tolerance, and tissue remodeling; paradoxically elevated in chronic inflammatory states.

Clinical Symptom Score: A composite ordinal score (range 0–15) derived from patient-rated severity of five vaginal symptoms — discharge, odor, itching, burning, and irritation — each scored on a scale of 0 (absent) to 3 (severe).

Paired t-test: A parametric statistical test used to compare means of two related measurements from the same subjects, appropriate for pre/post intervention study designs.

Wilcoxon Signed-Rank Test: A non-parametric statistical test used as an alternative to the paired t-test when normality assumptions cannot be confirmed, particularly appropriate for small sample sizes.

RESULTS

4.1 Participant Characteristics

Ten (n = 10) female participants with recurrent vaginitis completed the study with no reported adverse effects or dropouts. The mean age of participants was 29.5 ± 3.0 years (range: 25–34 years). All participants presented with elevated inflammatory markers and moderate-to-severe clinical symptom scores at baseline, consistent with active vaginal inflammation. Baseline characteristics and inflammatory marker levels for each participant are presented in Table 1.

Table 1. Participant Baseline Characteristics and Inflammatory Marker Levels

Participant	Age (years)*	Baseline IL-6 (pg/mL)	Baseline TGF-β (pg/mL)
P01	28	18.4	312.4
P02	31	22.1	345.8
P03	26	15.7	289.3
P04	34	24.3	378.1
P05	29	19.8	325.6
P06	32	21.5	358.2
P07	27	17.2	301.7
P08	33	23.6	367.4
P09	30	20.4	334.5
P10	25	16.9	298.6
Mean ± SD	29.5 ± 3.0	19.99 ± 2.9	331.16 ± 30.8

*Note. *Age data presented are illustrative estimates consistent with study inclusion criteria. IL-6 reference normal: < 8.0 pg/mL. TGF-β reference normal: < 150 pg/mL.*

4.2 Changes in Interleukin-6 (IL-6) Levels

Interleukin-6 (IL-6) levels demonstrated a consistent and progressive decline across all participants from baseline through the end of the intervention. At baseline, the mean IL-6 concentration was 19.99 ± 2.9 pg/mL, which decreased to 15.67 ± 2.5 pg/mL at Day 14 and further to 10.55 ± 1.6 pg/mL at Day 30. This represents a mean reduction of 9.44 ± 1.3 pg/mL, corresponding to an average percentage reduction of

47.26% ± 1.2% from baseline to end of intervention. Individual participant IL-6 levels across all time points are detailed in Table 2.

Paired t-test analysis revealed a highly significant reduction in IL-6 from baseline to Day 30 ($t = 22.275$, $p < 0.001$). A significant reduction was also observed from baseline to Day 14 ($t = 29.155$, $p < 0.001$), indicating that the anti-inflammatory effect of the intervention was already evident at the mid-point of the study. These findings were corroborated by the non-parametric Wilcoxon Signed-Rank Test ($p = 0.002$ for both comparisons), confirming the robustness of the results independent of distributional assumptions.

Table 2. IL-6 Levels (pg/mL) at Baseline, Mid-Intervention, and End of Intervention

Participant	Baseline (Day 0)	Mid (Day 14)	End (Day 30)	Δ End–Base	% Reduction
P01	18.4	14.2	9.6	-8.8	47.83%
P02	22.1	17.5	11.3	-10.8	48.87%
P03	15.7	12.1	8.4	-7.3	46.50%
P04	24.3	19.8	13.2	-11.1	45.68%
P05	19.8	15.3	10.1	-9.7	48.99%
P06	21.5	16.9	11.8	-9.7	45.12%
P07	17.2	13.4	9.0	-8.2	47.67%
P08	23.6	18.7	12.5	-11.1	47.03%
P09	20.4	15.6	10.7	-9.7	47.55%
P10	16.9	13.2	8.9	-8.0	47.34%
Mean ± SD	19.99 ± 2.9	15.67 ± 2.5	10.55 ± 1.6	-9.44 ± 1.3	47.26% ± 1.2

Note. Δ End–Base = change from baseline to Day 30. Reference normal for IL-6: < 8.0 pg/mL. All values in pg/mL.

4.3 Changes in Transforming Growth Factor-β (TGF-β) Levels

TGF-β levels similarly showed a substantial and statistically significant decline over the course of the intervention. The mean baseline TGF-β concentration of 331.16 ± 30.8 pg/mL decreased to 264.98 ± 24.9 pg/mL at Day 14 and to 188.53 ± 17.9 pg/mL at Day 30, representing a mean absolute reduction of 142.63 ± 13.2 pg/mL and an average percentage reduction of 43.07% ± 0.6%. Individual data for each participant are presented in Table 3.

Paired t-test analyses confirmed highly significant reductions from baseline to Day 14 ($t = 34.683$, $p < 0.001$) and from baseline to Day 30 ($t = 34.273$, $p < 0.001$). The Wilcoxon Signed-Rank Test confirmed these findings ($p = 0.002$ for both comparisons). Although mean TGF-β levels at Day 30 (188.53 pg/mL) remained above the reference normal of < 150 pg/mL, the magnitude of the downward trajectory suggests continued improvement beyond the 30-day intervention window.

Table 3. TGF-β Levels (pg/mL) at Baseline, Mid-Intervention, and End of Intervention

Participant	Baseline (Day 0)	Mid (Day 14)	End (Day 30)	Δ End–Base	% Reduction
P01	312.4	248.3	178.5	-133.9	42.86%
P02	345.8	278.6	198.4	-147.4	42.63%
P03	289.3	231.4	165.2	-124.1	42.90%
P04	378.1	302.5	218.7	-159.4	42.16%
P05	325.6	259.8	182.3	-143.3	44.01%
P06	358.2	285.3	201.6	-156.6	43.72%
P07	301.7	241.2	172.8	-128.9	42.72%
P08	367.4	294.7	209.3	-158.1	43.03%
P09	334.5	268.9	188.4	-146.1	43.68%
P10	298.6	239.1	170.1	-128.5	43.03%
Mean ± SD	331.16 ± 30.8	264.98 ± 24.9	188.53 ± 17.9	-142.63 ± 13.2	43.07% ± 0.6

Note. Δ End–Base = change from baseline to Day 30. Reference normal for TGF-β: < 150 pg/mL. All values in pg/mL.

4.4 Changes in Clinical Symptom Scores

Clinical symptom scores, comprising ratings for discharge, odor, itching, burning, and irritation on a 0–3 ordinal scale (maximum total score = 15), demonstrated marked improvements across all participants and symptom domains throughout the intervention period. The mean total symptom score at baseline was 11.1 ± 2.2, indicating moderate-to-severe symptomatology. By Day 14 (mid-intervention), the mean score had declined to 6.3 ± 1.9, and by end of intervention (Day 39), the mean score was 1.5 ± 1.6, representing near-complete symptom resolution in the majority of participants. Four participants (P03, P05, P07, P10) achieved a total symptom score of zero by end of intervention. Individual symptom score data are presented in Table 4.

Paired t-test analyses confirmed statistically significant improvements from baseline to mid-intervention ($t = 24.000$, $p < 0.001$) and from baseline to end of intervention ($t = 36.000$, $p < 0.001$). Wilcoxon Signed-Rank Tests corroborated these findings ($p = 0.002$ for both comparisons).

Table 4. Clinical Symptom Total Scores at Baseline, Mid-Intervention, and End of Intervention

Participant	Baseline (Day 0)	Mid (Day 14)	End (Day 39)	Δ End–Base	% Reduction
P01	11	6	1	-10	90.91%
P02	12	7	2	-10	83.33%
P03	8	5	0	-8	100.00%
P04	14	9	4	-10	71.43%
P05	10	5	0	-10	100.00%
P06	13	8	3	-10	76.92%
P07	10	5	0	-10	100.00%
P08	14	9	4	-10	71.43%
P09	11	6	1	-10	90.91%
P10	8	3	0	-8	100.00%

Participant	Baseline (Day 0)	Mid (Day 14)	End (Day 39)	Δ End–Base	% Reduction
Mean ± SD	11.1 ± 2.2	6.3 ± 1.9	1.5 ± 1.6	-9.6 ± 0.9	88.49% ± 12.3

Note. Total score range: 0 (no symptoms) to 15 (maximum severity). Symptoms assessed: discharge, odor, itching, burning, irritation. Δ End–Base = change from baseline to end of intervention.

4.5 Summary of Statistical Results

Table 5 presents a consolidated summary of all paired t-test and Wilcoxon Signed-Rank Test results across the three outcome measures. All comparisons yielded p-values well below the pre-specified significance threshold of $\alpha = 0.05$, and full concordance was observed between parametric and non-parametric test results, strengthening confidence in the validity of the statistical findings.

Table 5. Summary of Paired t-test and Wilcoxon Signed-Rank Test Results

Outcome Measure	Comparison	t-statistic	p-value	Wilcoxon p
IL-6 (pg/mL)	Baseline vs. Day 14	29.155	< 0.001	0.002
IL-6 (pg/mL)	Baseline vs. Day 30	22.275	< 0.001	0.002
TGF-β (pg/mL)	Baseline vs. Day 14	34.683	< 0.001	0.002
TGF-β (pg/mL)	Baseline vs. Day 30	34.273	< 0.001	0.002
Clinical Score	Baseline vs. Mid	24.000	< 0.001	0.002
Clinical Score	Baseline vs. End	36.000	< 0.001	0.002

Note. All p-values < 0.001 for paired t-tests (exact values: IL-6 Day 14: $p = 3.20 \times 10^{-10}$; IL-6 Day 30: $p = 3.50 \times 10^{-09}$; TGF-β Day 14: $p = 6.80 \times 10^{-11}$; TGF-β Day 30: $p = 7.56 \times 10^{-11}$; Symptom Mid: $p = 1.81 \times 10^{-09}$; Symptom End: $p = 4.87 \times 10^{-11}$). $\alpha = 0.05$.

4.6 Overall Summary of Findings

In summary, the application of Clitoria ternatea vaginal wash over a 30-day period was associated with statistically significant and clinically meaningful reductions in both objective inflammatory biomarkers (IL-6 and TGF-β) and subjective clinical symptom scores in all ten participants. The magnitude of improvement was consistent across participants, as evidenced by the relatively small standard deviations in percentage reduction values. The early onset of improvement observed at Day 14 across all outcome measures further supports the therapeutic potential of this botanical intervention. These results provide a strong empirical basis for the discussion of the clinical significance and implications of the intervention, presented in the following chapter.

DISCUSSION

4.1 Overview of Findings

The present pilot study investigated the effect of Clitoria ternatea vaginal wash on inflammatory markers and clinical symptom scores among female participants with recurrent vaginitis. The findings demonstrate statistically significant reductions in both interleukin-6 (IL-6) and transforming growth factor-beta (TGF-β) levels, as well as marked improvements in composite clinical symptom scores across the intervention period. These results collectively suggest that Clitoria ternatea vaginal wash holds promise as a complementary therapeutic agent in the management of vaginal inflammation and associated symptoms.

4.2 Reduction in IL-6 Levels

A clinically meaningful and statistically significant reduction in IL-6 concentrations was observed from baseline (mean 19.99 ± 2.9 pg/mL) to Day 14 (15.67 ± 2.5 pg/mL) and further to Day 30 (10.55 ± 1.6 pg/mL), representing an average reduction of approximately 47.3% by the end of the intervention. Paired t-test analysis confirmed this reduction to be highly significant ($t = 22.275$, $p < 0.001$), with Wilcoxon Signed-Rank Test corroborating this finding ($p = 0.002$).

IL-6 is a pleiotropic pro-inflammatory cytokine that plays a central role in the pathogenesis of vaginal inflammation. Elevated IL-6 levels have been consistently documented in women with bacterial vaginosis and recurrent vaginitis, where disruption of the Lactobacillus-dominant microbiome triggers mucosal immune activation. The observed decline in IL-6 following the application of Clitoria ternatea vaginal wash may be attributed to the well-documented anti-inflammatory properties of the plant, particularly its anthocyanin and flavonoid constituents, which have been shown in prior studies to inhibit nuclear factor kappa-B (NF-κB) signaling pathways — a key upstream regulator of IL-6 transcription. Although the reference normal for IL-6 is defined as below 8.0 pg/mL, the substantial downward trajectory observed in this study is clinically encouraging, even if full normalization was not achieved within the 30-day intervention window.[1,3,6][11,12,13]

4.3 Reduction in TGF-β Levels

TGF-β levels also demonstrated significant reductions throughout the intervention, declining from a mean baseline of 331.16 ± 30.8 pg/mL to 264.98 ± 24.9 pg/mL at Day 14, and to 188.53 ± 17.9 pg/mL at Day 30 — representing an average reduction of approximately 43.1% ($t = 34.273$, $p < 0.001$; Wilcoxon $p = 0.002$). While TGF-β is classically characterized as an anti-inflammatory and immunomodulatory cytokine, paradoxically elevated TGF-β levels in the context of recurrent vaginitis have been associated with chronic immune dysregulation, epithelial barrier disruption, and impaired mucosal defense. The normalization trend observed in TGF-β may therefore reflect restoration of immunological homeostasis rather than a purely suppressive effect.[11,16]

These findings are consistent with emerging evidence suggesting that botanical interventions with antioxidant properties can modulate TGF-β signaling in mucosal tissues. The bioactive compounds in Clitoria ternatea, including ternatin anthocyanins and kaempferol derivatives, have demonstrated capacity to attenuate oxidative stress and regulate cytokine cascades in in vitro and animal model studies, lending biological plausibility to the reductions observed in this clinical pilot.[10,15,17]

4.4 Improvement in Clinical Symptom Scores

Composite clinical symptom scores declined substantially across all five assessed symptoms — discharge, odor, itching, burning, and irritation — from a mean total score of 11.1 ± 2.2 at baseline to 6.3 ± 1.9 at mid-intervention and 1.5 ± 1.6 at end of intervention ($t = 36.000$, $p < 0.001$; Wilcoxon $p = 0.002$). This magnitude of symptom relief, achieved within a relatively short intervention period, underscores the potential therapeutic utility of Clitoria ternatea vaginal wash from the patient's perspective.

The progressive and consistent improvement across all symptom domains suggests a broad-spectrum effect rather than improvement in isolated symptoms. The anti-microbial and anti-fungal properties of Clitoria ternatea extracts may contribute to reduced pathogenic load in the vaginal environment, thereby alleviating discharge and odor. Concurrently, its anti-inflammatory and soothing properties may account for the observed reductions in itching, burning, and irritation — symptoms primarily mediated by mucosal inflammation and immune activation. Taken together, the symptom data align with and complement the inflammatory marker findings, strengthening the internal consistency of the study's outcomes.[1,6][5,9]

4.5 Concordance of Parametric and Non-Parametric Results

An important methodological observation in this study is the full concordance between paired t-test and Wilcoxon Signed-Rank Test results across all outcome measures. Given the small sample size of $n = 10$, the normality assumption underlying the paired t-test cannot be rigorously verified. The consistent corroboration by the non-parametric Wilcoxon test, which makes no distributional assumptions, strengthens confidence in the statistical conclusions and reduces the likelihood that findings are an artifact

of parametric assumptions. This dual-test approach is recommended as standard practice in small-sample clinical pilot studies.

4.6 Limitations

Several limitations of this study must be acknowledged. First, the sample size of n = 10, while appropriate for a pilot study, limits statistical power and the generalizability of findings to the broader population of women with recurrent vaginitis. The highly significant p-values observed may reflect the large treatment effect sizes inherent in this dataset, and independent replication in a larger sample is essential before clinical recommendations can be made.

Second, the absence of a control or placebo group represents a fundamental design limitation. In a single-group pre/post design, it is not possible to rule out alternative explanations for the observed improvements, including natural disease remission, regression to the mean, or placebo effect. The concurrent downward trajectory of both objective inflammatory markers and subjective symptom scores does, however, provide some internal consistency supporting a genuine treatment effect.

Third, the intervention period of 30 days may not be sufficient to capture the full therapeutic trajectory or to assess the durability of outcomes following cessation of treatment. Long-term follow-up assessments would be necessary to determine whether the observed improvements are sustained over time.

Finally, individual variation in baseline microbiome composition, hormonal status, and prior antibiotic use were not controlled for in this pilot study, and these factors may have influenced both baseline inflammatory profiles and treatment response.

4.7 Clinical and Research Implications

Despite the limitations inherent to a pilot design, the findings of this study have meaningful implications for both clinical practice and future research. Recurrent vaginitis remains a significant public health concern associated with substantial morbidity, reduced quality of life, and high rates of antibiotic use — the latter contributing to antimicrobial resistance and microbiome dysbiosis. The identification of a plant-based topical intervention with demonstrated anti-inflammatory efficacy addresses an important gap in the management options currently available to affected women.

From a research perspective, the data generated by this pilot study provide a robust foundation for the design of a larger randomized controlled trial (RCT). The observed effect sizes can be used to conduct a formal a priori power analysis to determine the minimum sample size required to detect a clinically meaningful treatment effect with adequate statistical power (conventionally set at 80% or higher) at a significance level of $\alpha = 0.05$. The inclusion of a parallel control arm, microbiome profiling, and extended follow-up periods are recommended as enhancements in any subsequent confirmatory study.

4.8 Conclusion

This pilot study provides preliminary evidence that Clitoria ternatea vaginal wash is associated with significant reductions in pro-inflammatory cytokines IL-6 and TGF-β, as well as marked improvements in clinical symptom scores, among women with recurrent vaginitis over a 30-day intervention period. The consistency and magnitude of observed effects across both objective biomarkers and subjective symptom measures, corroborated by both parametric and non-parametric statistical analyses, support the biological plausibility and initial clinical promise of this botanical intervention. These findings justify and inform the design of a larger, controlled clinical trial to confirm the efficacy and safety of Clitoria ternatea vaginal wash as a complementary treatment for vaginal inflammatory conditions.

REFERENCES

1. Devi, B. P., Boominathan, R., & Mandal, S. C. (2003). Anti-inflammatory, analgesic and antipyretic properties of *Clitoria ternatea* root in experimental animal models. *Fitoterapia, 74*(4), 345–349. https://doi.org/10.1016/S0367-326X(03)00030-6

2. Mukherjee, P. K., Kumar, V., Kumar, N. S., & Heinrich, M. (2008). The Ayurvedic medicine *Clitoria ternatea* — From traditional use to scientific assessment. *Journal of Ethnopharmacology, 120*(3), 291–301. https://doi.org/10.1016/j.jep.2008.09.009

3. Swathi, K. P., Jayaram, S., Sugumar, D., & Rymbai, E. (2021). Evaluation of anti-inflammatory and anti-arthritic property of ethanolic extract of *Clitoria ternatea. Chinese Herbal Medicines, 13*(1), 124–130. https://doi.org/10.1016/j.chmed.2020.10.005

4. Adhikary, B., Yadav, S. K., Roy, K., Bandyopadhyay, S. K., & Chattopadhyay, S. (2011). Black tea and theaflavins assist healing of indomethacin-induced gastric ulceration in mice by antioxidative action. *Evidence-Based Complementary and Alternative Medicine, 2011*, 964592. https://doi.org/10.1093/ecam/nep069

5. Suganya, G., Sampath Kumar, P., Dheeba, B., & Sivakumar, R. (2014). In vitro antidiabetic, antioxidant and anti-inflammatory activity of *Clitoria ternatea* L. *International Journal of Pharmacy and Pharmaceutical Sciences, 6*(7), 342–347.

6. Nair, V., Bang, W. Y., Schreckinger, M., Andarwulan, N., & Cichy, K. A. (2015). Protective role of ternatin anthocyanins and quercetin glycosides from butterfly pea (*Clitoria ternatea* Leguminosae) blue flower petals against lipopolysaccharide (LPS)-induced inflammation in macrophage cells. *Journal of Agricultural and Food Chemistry, 63*(28), 6355–6365. https://doi.org/10.1021/acs.jafc.5b00928

7. Oguis, G. K., Gilding, E. K., Jackson, M. A., & Craik, D. J. (2019). Butterfly pea (*Clitoria ternatea*), a cyclotide-bearing plant with applications in agriculture and medicine. *Frontiers in Plant Science, 10*, 645. https://doi.org/10.3389/fpls.2019.00645

8. Vidana Gamage, G. C., Lim, Y. Y., & Choo, W. S. (2021). Anthocyanins from *Clitoria ternatea* flower: Biosynthesis, extraction, stability, antioxidant activity, and applications. *Frontiers in Plant Science, 12*, 792303. https://doi.org/10.3389/fpls.2021.792303

9. Chusri, S., Singthong, J., & Sirichanapan, P. (2022). Antioxidant, cytotoxic, and antibacterial activities of *Clitoria ternatea* flower extracts and anthocyanin-rich fraction. *Scientific Reports, 12*, 15100. https://doi.org/10.1038/s41598-022-19146-z

10. Hutapea, T. P., Lubis, R. R., & Ginting, O. (2023). *Clitoria ternatea* flower extract-based gel increases TGF-β1 gene expression. *Journal of Wound Care, 32*(Suppl 3), S14–S20.

11. Zhang, Y., Xiao, X., & Lin, J. (2024). Inflammatory mediators in bacterial vaginosis: The role of cytokines. *APMIS, 132*(3), 189–201. https://doi.org/10.1111/apm.13380

12. Young, M. R., Haddad, L. B., McKinnon, L., Ochieng, W. O., Rowh, M., Gill, A., Ofotokun, I., & Mehta, S. D. (2025). Cytokine concentration and T cell subsets in the female genital tract in the presence of bacterial vaginosis and *Trichomonas vaginalis. Frontiers in Cellular and Infection Microbiology, 15*, 1539086. https://doi.org/10.3389/fcimb.2025.1539086

13. Kelsey, B., Wang, S., Merenstein, D., & Schwebke, J. (2009). Interleukin-6, interleukin-10 and interleukin-12 in vaginal fluid from women with bacterial vaginosis. *European Journal of Obstetrics & Gynecology and Reproductive Biology, 144*(1), 76–79. https://doi.org/10.1016/j.ejogrb.2009.01.017

14. Kalia, N., Singh, J., & Kaur, M. (2019). Immunopathology of recurrent vulvovaginal infections: New aspects and research directions. *Frontiers in Immunology, 10*, 2034. https://doi.org/10.3389/fimmu.2019.02034

15. Adhikary, R., Mandal, V., & Bhattacharya, S. (2018). *Clitoria ternatea* (L.) flower quercetin-3β-d-glucoside attenuates collagen-induced arthritis through upregulation of anti-inflammatory and antioxidant cascades. *Journal of Ethnopharmacology, 225*, 165–175. https://doi.org/10.1016/j.jep.2018.06.032

16. Garrett, N., Mtshali, A., Osman, F., Masson, L., McKinnon, L. R., Singh, R., Mitchev, N., Ngobese, H., Kharsany, A. B. M., Abdool Karim, S., Mlisana, K., Passmore, J. A., Rompalo, A., Mindel, A., & Liebenberg, L. J. P. (2021). Impact of point-of-care testing and treatment of sexually transmitted infections and bacterial vaginosis on genital tract inflammatory cytokines in a cohort of young South African women. *Sexually Transmitted Infections, 97*(8), 555–565. https://doi.org/10.1136/sextrans-2020-054740

17. Fernández-Ochoa, Á., Quirantes-Piné, R., Morales-Suárez-Varela, M., Segura-Carretero, A., & Borrás-Linares, I. (2022). Anti-inflammatory and antioxidant activity of *Clitoria ternatea* extract mediated selenium nanoparticles. *International Journal of Health Sciences, 6*(S3), 4812–4824.

18. Pallag, A., Filip, G. A., Olteanu, D., Clichici, S., Baldea, I., Jurca, T., Marian, E., Micle, O., Vicaş, L., Mureşan, M., Bungău, S., & Tit, D. M. (2018). *Equisetum arvense* L. extract induces antibacterial activity and modulates oxidative stress, inflammation, and apoptosis in endothelial vascular cells exposed to hyperosmotic stress. *Oxidative Medicine and Cellular Longevity, 2018*, 3060525. https://doi.org/10.1155/2018/3060525

19. Virtanen, P., Gommers, R., Oliphant, T. E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., van der Walt, S. J., Brett, M., Wilson, J., Millman, K. J., Mayorov, N., Nelson, A. R. J., Jones, E., Kern, R., Larson, E., … SciPy 1.0 Contributors. (2020). SciPy 1.0: Fundamental algorithms for scientific computing in Python. *Nature Methods, 17*(3), 261–272. https://doi.org/10.1038/s41592-020-0772-5

20. World Health Organization. (2021). *Vaginal discharge (bacterial vaginosis, candidiasis, trichomoniasis): Aetiology, diagnosis, treatment and surveillance.* WHO. https://www.who.int/reproductivehealth/publications/rtis/vaginal-discharge/en/