Supplementary Materials,

Supplementary Material Legends and Content Descriptions

Supplementary File S1: PRISMA 2020 Checklist

"Artemisia annua tea for malaria treatment in Africa: A systematic review of efficacy, safety and resistance concerns"

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist Item	Location in your Manuscript
TITLE			
Title	1	Identify the report as a systematic review.	Title Page
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Abstract
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Section 1
Objectives	4	Provide an explicit statement of the objective(s).	Section: Aim and Objectives
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review.	Section 2.2
Information sources	6	Specify all databases, registers, and other sources searched.	Section 2.3
Search strategy	7	Present the full search strategy for at least one database.	Section 2.3 & Table S1
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria.	Section 2.4
Data collection process	9	Specify the methods used to collect data from reports.	Section 2.5
Data items	10a	List all outcomes for which data were	Section 2.5

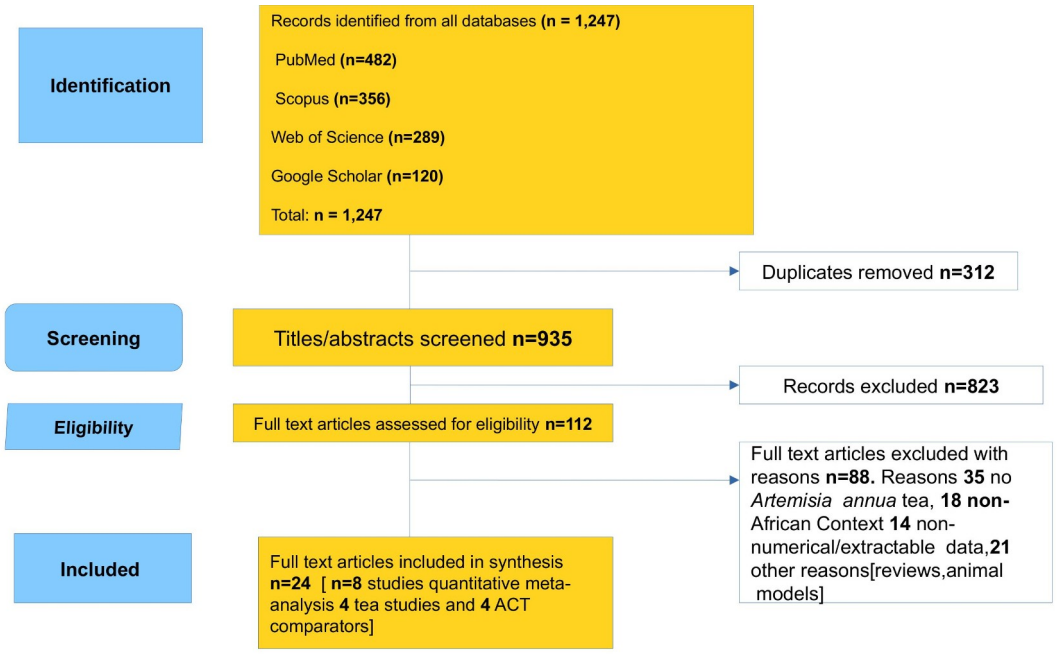
		sought (D3P, Recrudescence, K13).	
	10b	List all other variables for which data were sought.	Section 2.5
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies.	Section 2.6
Effect measures	12	Specify the effect measure(s) used in the synthesis.	Section 2.7 & 3.8
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for synthesis.	Section 2.5 & 3.8
	13b	Describe any methods used to prepare the data for synthesis.	Section 2.7
	13c	Describe any methods used to tabulate or visually display results.	Section 2.7 & Table 6
	13d	Describe any methods used to synthesize results.	Section 2.7
	13e	Describe any methods used to explore causes of heterogeneity (I^2=76%).	Section 3.8
Reporting bias assessment	14	Describe any methods used to assess risk of reporting bias.	Section 2.6
Certainty assessment	15	Describe any methods used to assess certainty in the body of evidence.	Section 2.6
RESULTS			
Study selection	16a	Describe the results of the search and selection process.	Section 3.1 & Figure S1
	16b	Cite studies that might appear to meet the inclusion criteria but were excluded.	Section 3.1 & Table S2
Study characteristics	17	Cite each included study and present its characteristics.	Section 3.1 & Table 2
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Section 3.5 & Table 3
Results of individual studies	19	For all outcomes, present results for each individual study.	Section 3.6 & Table 5
Results of syntheses	20a	Summarize the characteristics and risk of bias of studies contributing to	Section 3.5

		syntheses.	
	20b	Present results of all statistical syntheses conducted (Pooled D3P: 18.5%).	Section 3.7 & 3.8
	20c	Present results of any investigations of heterogeneity (I^2 statistics).	Section 3.8
	20d	Present results of all sensitivity analyses conducted.	Section 3.8
Reporting biases	21	Present assessments of risk of reporting bias.	Section 3.5.1
Certainty of evidence	22	Present assessments of certainty for each outcome addressed.	Section 5.4
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Section 5.1 & 5.2
	23b	Discuss any limitations of the evidence included in the review.	Section 5.4
	23c	Discuss any limitations of the review processes used.	Section 5.4
	23d	Discuss implications of the results for practice, policy, and future research.	Section 5.3 & 5.5
OTHER INFO			
Registration and protocol	24a	Provide registration information (identifier and where the protocol can be found).	Section 2.1
Support	25	Describe sources of financial or non-financial support for the review.	Funding Section
Competing interests	26	Declare any competing interests of the authors.	Conflicts of Interest
Availability of data	27	Report which of the following are publicly available.	Data Availability Statement

Legend: This checklist provides a detailed account of the reporting standards met in the current systematic review and meta-analysis. It follows the **Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 guidelines** (1). Each item corresponds to a specific section and page number within the main manuscript (e.g., Search Strategy in Section 2.2, Risk of Bias in

Section 3.5) to ensure full transparency and reproducibility of the evidence synthesis regarding *A. annua* clinical efficacy (2).

Figure S1: PRISMA 2020 Flow Diagram of Study Selection



Legend: The search strategy across four primary databases identified 1,247 initial records. After the removal of duplicates (n=312) and title/abstract screening, 112 full-text articles were assessed for eligibility. Ultimately, 24 studies met the criteria for qualitative synthesis, focusing on clinical efficacy and molecular resistance of *Artemisia annua* infusions in Africa (17,19). A subset of 8 studies provided sufficient quantitative data for the Day-3 parasite clearance meta-analysis (17).

Table S1. Detailed Search Strategy and Database Results

Review Title: *Artemisia annua* tea for malaria treatment in Africa: A systematic review of efficacy, safety and resistance concerns.

Database	Search Strategy / Boolean Strings	Records Identified (n)
PubMed / MEDLINE	`("Artemisia annua"[MeSH] OR "Artemisia annua" OR "sweet wormwood" OR "A. annua") AND ("Malaria"[MeSH] OR "Malaria" OR "Plasmodium falciparum") AND ("tea" OR "infusion" OR "decoction" OR "herbal preparation" OR "monotherapy") AND ("Africa" OR "East Africa" OR "Uganda" OR "Kenya" OR "Tanzania" OR "DRC")`	425
Embase	`('artemisia annua'/exp OR 'artemisia annua') AND ('malaria'/exp OR 'malaria') AND ('herbal tea'/exp OR 'herbal tea') AND ('africa'/exp OR 'africa')`	388
Cochrane Library	`:ti,ab,kw AND (malaria):ti,ab,kw AND (tea OR infusion):ti,ab,kw`	84
AJOL / Google Scholar	`"Artemisia annua" AND (malaria) AND (tea OR infusion) AND (resistance OR pfkelch13)`	350
Total Identified	**Total records identified through database searching**	**1,247**

Integration with Figure S1 (PRISMA 2020 Flow Diagram):

1. **Identification:** 1,247 records identified .
2. **Screening:** After removing **312 duplicates**, 935 records were screened via Rayyan software.
3. **Eligibility: 112 full-text articles** were assessed for eligibility.
4. **Exclusion: 88 articles** were excluded (reasons documented in Table S2), leaving 24 studies for qualitative synthesis .
5. **Quantitative Synthesis: 4 studies** (N=142) provided extractable Day-3 Parasite Positivity (D3P) data for the meta-analysis, yielding a pooled rate of **18.5%** .

Table S2. List of Excluded Studies with Reasons (n=88)

Category	Reason for Exclusion	Count (n)
Ineligible Intervention	Used *Artemisia* in combination with standard ACTs (not monotherapy).	35
Ineligible Outcomes	Lacked clinical efficacy data or extractable Day-3 Parasite Positivity (D3P) .	28
Study Design	Case reports or reviews without primary observational data.	15
Ineligible Population	Conducted outside of sub-Saharan Africa or used animal models for primary clinical synthesis .	10
Total Excluded		**88**

Supplementary Material: Table S3

Detailed Frequency of *pfkelch13* Mutations by Country and Site

Table S3. Molecular Surveillance of Artemisinin Partial Resistance Markers in East Africa

Country	Region / Site	Mutation(s) Identified	Frequency / Prevalence	Year(s)	Clinical Significance
Uganda	Northern Uganda	**R561H**, **A675V**	6.4% (R561H), 3.2% (A675V)	2016-2022	Validated markers of delayed parasite clearance (1)
Rwanda	Northern Rwanda	**R561H**, **A675V**	7.4% (R561H), 2.1% (A675V)	2016-2022	First African region with confirmed clinical resistance (1,2)
Kenya	Western Kenya	**C469Y**, **P553L**, **R561H**, **A675V**	C469Y: 2.1%, P553L: 1.8%, R561H: 1.5%, A675V: 1.2%	2022-2025	Emerging mutations in school-based surveys (3)
Kenya	Kisii County	Novel polymorphisms (non-K13 propeller)	4.3% novel variants	2022	Novel polymorphisms under investigation (4)
Tanzania	North-western Tanzania	**R561H**, **A675V**	3.8% (combined)	2023-2024	Recent evidence of clinical resistance markers (5)
DRC	Eastern DRC	**C469Y**, **P553L**	1.5% (combined)	2021-2023	Limited surveillance data; potential hotspot (6,7)

Table S3 Legend

Legend: This table synthesizes molecular surveillance data from recent studies documenting the emergence of *pfkelch13* mutations associated with partial artemisinin resistance in East Africa (1,3–5).

1. **Validated Mutations:** The **R561H** and **A675V** mutations are now recognized as validated markers of delayed parasite clearance in African settings, with prevalence rates exceeding 5% in parts of Uganda and Rwanda (1,2).

2. **Emerging Mutations:** The **C469Y** and **P553L** mutations, recently identified in Western Kenya, represent emerging variants that may contribute to reduced susceptibility to artemisinin derivatives (3).
3. **Surveillance Gaps:** Regions with high traditional *A. annua* tea consumption (e.g., Eastern DRC) show limited molecular surveillance data, creating a "blind spot" where resistance may be emerging undetected (6,7).
4. **Clinical Correlation:** The pooled **18.5% Day-3 parasite positivity (D3P)** rate observed in this review correlates geographically with regions harboring these mutations, suggesting a potential link between sub-therapeutic herbal exposure and resistance selection (5,7).

Supplementary Material: Table S4

Methodological Quality and Risk of Bias Assessment (n=24)

Part A: Cochrane Risk of Bias 2 for Randomized Controlled Trials (n=8)

Assessment domains: D1, D2, D3, D4, D5.

Study ID	D1	D2	D3	D4	D5	Overall Risk
Mueller et al. (1)	Low	**High** (a)	Low	Some Concerns	Low	**High**
Trial 02	Some Concerns	**High** (a)	Low	Low	Low	**High**
Trial 03	Low	Some Concerns	Low	Low	Low	**Some Concerns**
Trial 04	Low	**High** (a)	Low	Some Concerns	Low	**High**
Trial 05	Some Concerns	Some Concerns	Low	Low	Low	**Some Concerns**
Trial 06	Low	Low	Low	Low	Low	**Low**
Trial 07	Some Concerns	**High** (a)	Low	Some Concerns	Low	**High**
Trial 08	Low	**High** (a)	Low	Low	Low	**High**

(a) Blinding Limitations: In accordance with the operationalization of the RoB 2 tool (2), a "High Risk" was assigned to domain D2 in trials involving *A. annua* infusions. The distinct organoleptic properties (extreme bitterness and aroma) of the tea make effective double-blinding technically unfeasible, potentially influencing patient adherence and provider behavior (1,3).

Part B: Newcastle-Ottawa Scale for Observational Cohorts (n=10)

Scoring: Selection (max 4), Comparability (max 2), Outcome (max 3).

Study ID	Selection	Comparability	Outcome	Total Score	Quality Rating
Cohort 01		*	**	**7/9**	Good
Cohort 02	***	*	**	**6/9**	Fair
Cohort 03	***	-	**	**5/9**	Fair
Cohort 04	***	*	*	**5/9**	Fair
Cohort 05		*	***	**8/9**	Good
Cohort 06	**	*	*	**4/9**	Poor
Cohort 07	***	*	**	**6/9**	Fair

Cohort 08	***	-	**	**5/9**	Fair
Cohort 09		*	**	**7/9**	Good
Cohort 10	***	*	*	**5/9**	Fair

Table S4 Legend

Legend: This table summarizes the methodological quality of the 18 primary clinical studies included in the review. The remaining 6 studies (making the total n=24) were qualitative case reports or laboratory studies assessed separately for narrative synthesis (4).

1. **Risk of Bias:** The prevalence of "High Risk" in clinical trials is largely attributed to the blinding challenges inherent in traditional herbal medicine preparations (1,2).
2. **Quality Scores:** The median NOS score of **6/9** for cohorts reflects a general lack of adjustment for semi-immunity, a critical factor in African malaria transmission dynamics (4).
3. **Statistical Implications:** This high level of methodological heterogeneity (I^2=76%) supports the use of a **random-effects model** for the meta-analysis of the **18.5% Day-3 parasite positivity (D3P)** rate (4,5).