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EFFICACY OF ANTI-MALARIAL HERBAL MEDICINES USED BY COMMUNITIES IN MALARIA AFFECTED REGIONS GLOBALLY: A SYSTEMATIC REVIEW, EVIDENCE AND GAP MAP PROTOCOL

Authors

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ABSTRACT

Introduction: With the rising resistance to Artemisinin Combination Therapies (ACTs), there is a need to hasten the discovery and development of newer anti-malarial agents. Herbal medicines are key for the development of novel drugs. Currently, herbal medicine usage in communities for treatment of malaria symptoms is common as an alternative to conventional anti-malarial medicine. However, the efficacy and safety of most of the herbal medicines has not yet been established. Therefore, this systematic review and evidence and gap map intend to collate and map the available evidence, identify the gaps, and synthesize the efficacy of herbal antimalarial medicines in malaria affected regions globally.

Methods and analysis: The systematic review and evidence and gap map (EGM) will be done following PRISMA and Campbell Collaboration guidelines respectively. This protocol has been registered in PROSPERO. Data sources will include PubMed, MEDLINE Ovid, EMBASE, Web of Science, Google Scholar, and grey literature search. Data extraction will be done in duplicate using a data extraction tool tailored in Microsoft Office excel for herbal antimalarials discovery research questions following the PICOST framework. The Risk of Bias and overall quality of evidence will be assessed using Cochrane risk of bias tools and GRADE approaches. Data analysis will be done using both structured narrative and quantitative synthesis. The primary review outcomes will be clinically important efficacy and adverse drug reactions. Laboratory parameters will include IC_{50}, $RSA_{0\text{-}3h}$, TSA_{50} and parasite clearance rate.

Results: The findings of this review will help map the use of herbal medicines in communities for management of malaria symptoms; identify the gaps in the evidence for efficacy of antimalarial herbals and inform new antimalarial drug discovery research.

Ethics and dissemination: The review protocol was approved by the School of Biomedical Science Research Ethics Committee, Makerere University College of Health Sciences (SBS-2022-213).

PROSPERO Registration Number: CRD42022367073

Key words: Herbal medicines, Antimalarial, Plasmodium falciparum, malaria, herbal extracts

Article summary

Strengths and limitations of this study

Strength

- The review will provide an evidence gap map on use of herbal antimalarial agents for malaria management in communities affected by malaria globally.
- The review will provide an update on efficacy of herbal products against malaria parasites.
- The study will combine both evidence synthesis and evidence gap map on herbal antimalarial products in malaria affected regions.

Limitations

- The review will focus only on herbal medicines whose efficacy has been compared against artemisinin agents as these are the current cornerstone in malaria treatment

INTRODUCTION

Malaria remains a global public health problem affecting nearly half of the world's population. In the year 2020, global estimates indicated 241 million malaria cases and 627 000 malaria deaths (1). Malaria was still deadly in 2021 as the 6th most important cause of death in Africa ahead of COVID-19 that was the 7th, up from the 22nd in 2020 driven by the *delta* variant. Sub-Saharan Africa carries the greatest burden, accounting for about 95% of all malaria cases and 96% of all deaths in 2020 (1)

Chemotherapy with effective antimalarial medicines remains the most predominant intervention for effective management of malaria globally (2). Artemisinin-based combination therapies (ACTs) are the main stay and frontline treatments for uncomplicated *Plasmodium falciparum* malaria (3). However, malaria treatment faces a number of challenges globally including; drug resistance, poor quality medicines, inaccessibility, unavailability and high cost (4). Resistance to ACTs was originally reported in areas of the Greater Mekong Sub region (GMS) such as Thailand and Cambodia (5). Currently, artemisinin resistance has also independently arisen in East Africa (Rwanda and Uganda) (5). This is a major threat for the global initiative on elimination and eradication of malaria (6) justifying concerted efforts to hasten the discovery and development of newer anti-malarial molecules.

Traditional medicines (herbs) remain the corner stone for the discovery of novel drugs with more desirable medicinal and pharmacological properties (7, 8). Use of traditional medicine for treatment of malaria symptoms has previously been achieved with *Quinghao* isolated from a Chinese herbal medicine, *Artemisia annua* and quinine isolated from *Cinchona* species (Rubiaceae) (9, 10). n various malaria endemic regions globally, plants are traditionally used to alleviate fevers and treat malaria. This is due to inherited cultural practices and belief in traditional medicine, accessibility and relatively lower costs compared to the modern medicines, largely unknown and underappreciated toxicities and perceived efficacy (11).

Why this review?

Communities have now resorted to use of natural plant products (herbs) that are assumed or claimed to have antimalarial efficacy. With the prevailing challenges facing the use of ACTs, use of herbal products and development of alternative conventional antimalarial medicines is key for malaria control and eradication efforts globally. However, the antimalarial efficacy of most herbal agents used by communities in malaria affected regions remains unknown. The continued use of agents with unproven efficacy is potentially harmful to the population as it can lead to unwanted outcomes such as delay in getting effective treatment, morbidity, and mortality. There is thus need, to establish the comparative antimalarial efficacy of herbal medicines used in malaria affected regions globally and the artemisinin agents. This will help guide the current efforts to develop alternative antimalarial agents globally.

Several reviews have been conducted documenting information on different plants for their anti-plasmodial and antimalarial activities. (11-13). However, these reviews focused on studies that investigated *In-vitro* and *In-vivo* activities of these herbs without comparing their efficacies with the already existing modern anti-malarial medicines. Our current review seeks to collate and map evidence comparing the antimalarial efficacy of herbal medicines with that of artemisinin agents in malaria affected regions globally.

How the intervention might work

Early detection and treatment remain the principal strategies for control of malaria globally. Treatment of malaria currently involves the use artemisinin combination therapies as recommended by WHO globally. However, this strategy is affected by the rising levels of drug

resistance to artemisinin and the partner drugs and the relatively high cost. Artemisinin agents are the current cornerstone in treatment of malaria globally however, they are faced with the threat of resistance development. Herbal medicines with anti-plasmodial activity are used by communities in treatment of malaria in malaria affected regions globally. They therefore provide an alternative to artemisinin agents in malaria affected regions globally. Efficacious herbal medicines provide activity against both sensitive and slow artemisinin clearing parasites. Herbal antimalarial products may also help improve access to malaria treatment due to the cheaper cost, and convenient dosing. Thus, potentially contributing to the anti-malarial drug development pipeline.

METHODS

This will be a multi-modal blended systematic review and evidence gap map. The evidence and gap map and systematic review will be done following the Campbell Collaboration (14) and Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines (15). This protocol was registered in PROPSERO (https://www.crd.york.ac.uk/prospero/) registration number CRD42022367073 (16)

Intervention-outcome framework for the evidence and gap map

The intervention domains will include medicinal plants and plant extracts. There will be two outcome domains clinical (symptom resolution; fever, body aches/fatigue, malaise, joint pains, nausea/vomiting, loss of appetite) and laboratory (IC_{50}, $RSA_{0\text{-}3hrs}$, TSA_{50}, parasite clearance rate) (Table 1).

Table 1: Intervention-outcome domains for the evidence and gap map

Intervention Domains	***Indicators***	***Description***
• *Medicinal plants*	• Kind/type of plant (shrub, herb, tree)	• The type of plant, including geographical location
	• Parts of the plant used (leaves, back, roots, shoots)	• Plant material used, either entire plant or its parts (single or combined)
	• Name of the plant species	• Include both Genus and species names
	• Age of the plant/part	• Either young or mature/old plant/or parts
• *Plant extract*	• Water extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Methanolic extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Ether extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Chloroform extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Methanol extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Ethanolic extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Crude extract	• Proportions of the different extracts in the mixture
Outcomes Domains	Indicators	Description
• *Clinical outcomes*	• Fever	• Relief from fever
	• Body aches/malaise	• Relief from body aches
	• Joint pains	• Relief from joint pains
	• Nausea/vomiting	• Relief from vomiting
	• Appetite	• Gaining of appetite
• *Laboratory outcomes*	• IC_{50},	• Inhibitory concentration killing 50% of parasites
	• $RSA_{0\text{-}3hrs}$	• Concentration killing early-stage parasites (0-3hrs)
	• TSA_{50}	• Concentration killing 50% of trophozoites

Review question

What is the *In-vitro* and *In-vivo* efficacy of anti-malarial herbal medicines used by communities in malaria affected regions globally?

Table 2: PICOST model for the review question

Element of PICO	Description
Population (P)	Clinical: Malaria patients (children and adults) Laboratory: Malaria parasites, *Plasmodium spp (e.g: P. falciparum).* Wild or field strain parasites
Intervention/Exposure	Clinical: Use of herbal medicines for management of symptoms of malaria among children and adult patients Lab: Plant extracts used in *in-vitro* assays against *Plasmodium* parasites
Comparator	• *In-vitro (laboratory) studies:* Artemisinin antimalarial agents • *In-vivo (clinical) studies:* None
Outcomes	Clinical: symptom resolution (fever, nausea/vomiting, joint pains, fatigue/malaise, loss of appetite) Laboratory: IC_{50}, $RSA_{0\text{-}3h}$, TSA_{50}, parasite suppression rate
Setting	Malaria affected countries globally (tropical countries) Designs: Cross-sectional studies, Randomized controlled trials (RCTs)
Time	2000 to date (Introduction of Artemisinin in malaria treatment outside China)

Outcomes

Primary outcomes

- Clinical: symptom resolution (fever, nausea/vomiting, joint pains, fatigue/malaise, loss of appetite)
- Laboratory: IC_{50}, $RSA_{0\text{-}3h}$, TSA_{50}, and parasite suppression rate

Secondary outcome

- Rate of symptom resolution will be the safety of the anti-malarial herbal medicines
- Safety of antimalarial herbal medicines

Eligibility criteria

Inclusion criteria

- Peer reviewed articles reporting clinical efficacy of herbal medicines in malaria treatment among children and adults in malaria affected regions globally
- Peer reviewed articles reporting on herbal medicinal plants used by communities for management of symptoms of malaria in malaria affected regions globally
- Peer reviewed articles reporting on the safety of herbal medicines used by communities in management of malaria symptoms
- Peer reviewed articles reporting on *In-vitro* efficacy of anti-malarial herbal medicines
- Peer reviewed articles published from the years 2000 to date
- Peer reviewed articles published in English

Exclusion criteria

- Peer reviewed articles whose full text cannot be retrieved
- Peer reviewed articles from studies that did not receive ethical review and approval

Identification of articles

Data Sources

Article search will be performed by an experienced librarian in PubMed, MEDLINE Ovid, EMBASE and Web of Science, and other grey literature sources. Medical subject headings (MeSH) terms and Boolean operators "AND" and "OR" will be used to search for the articles. The data sources will include scholarly databases, grey literature sources, contacting authors as well as screening reference sources of included studies. We will also search Google Scholar for additional studies that may be missed from the other sources. The search will be limited to 2000 to 2022.

We will also screen through reference lists of included studies for additional eligible studies that may not be identified by the search. Bibliography search of the reference list of included articles will be screened for potential articles for inclusion in the review.

Search Strategy

The search terms below will be used to identify eligible studies, based on PICOST:

Population:

Laboratory: Plasmodium falciparum, malaria parasites, Plasmodium species, Plasmodium spp, wild type parasites, field parasites, field isolates

Clinical: Children, infants, adults

Disease/condition: Malaria, fever, malaria fever, malaria symptomatic patients, Plasmodium falciparum infection, Plasmodium infection

Intervention: Herbal medicine* OR herbal remed* OR medicinal plant* OR herbal formulation* OR plant medicine* OR herbal product* OR plant extract* OR medicinal herb* OR ethnomedicine* OR traditional herbal medicine OR alternative medicine OR ethnobotany OR phyto-medicine, shrub, herb, shoot, leaves, roots, herbal extract

Comparator:

Clinical: None

Laboratory: Dihydroartemisinin, Lumefantrine, Piperaquine

Outcome:

Clinical: Fever, nausea, vomiting, joint pains, malaise, loss of appetite, symptom resolution, symptom clearance

Laboratory: IC_{50}, $RSA_{0\text{-}3hrs}$, TSA_{50}, malaria parasite clearance, Plasmodium clearance, *Plasmodium falciparum* clearance, malaria parasite suppression rate

Setting: Malaria-affected AND (region* OR countr* OR area*) OR Africa OR Amazon region OR Bangladesh OR Burkina Faso OR Burundi OR Central African Republic OR Central America OR Chad OR Côte d'Ivoire OR Ivory Coast OR South America OR Cambodia OR Cameroon OR the Caribbean OR Democratic Republic of the Congo OR Grande Comoros OR Guinea OR Island OR Ethiopia OR Ghana OR Dominican Republic OR Equatorial Guinea OR Haiti OR India OR Indonesia OR Kenya OR Latin America OR Liberia OR Madagascar OR Malawi OR Mekong Delta Sub-region OR Mali OR Middle East OR Mozambique OR Myanmar OR Niger OR Nigeria OR Oceania OR Pacific islands OR Pakistan OR Rwanda OR Senegal OR Sierra Leone OR Solomon Islands OR Southeast Asia OR Sub-Saharan Africa OR Thailand OR Togo OR Uganda OR United Republic of Tanzania OR Venezuela OR Zambia OR Zimbabwe.

Time: 2000-2022, in line with the introduction of Artemisinin in malaria treatment.

Data management, screening, and selection

For the initial management of references from search results, EndNote software will be used. The articles will be exported to Endnote v20 and duplicates will be removed. The articles will then be screened in duplicate using predetermined eligibility criteria. The screening will be performed independently by the review team pair (KOO and LN) in EPPI-Reviewer v4.13.0.0, using a screening tool developed *a priori* and piloted using 10% of the search yield, any disagreements between the reviewers will be resolved by consensus, and any further disagreements will be referred to the tie breaker (OM).

Data abstraction and coding

The data abstraction tool will be created and piloted using 10% of the eligible studies to ensure it captures all relevant data from included studies and the uploaded on EPPI-Reviewer v4.13.0.0. The coding process will be carried out independently by two research team members (KOO and LN), whose results will be reconciled and disagreements resolved through discussion, and their results will later be validated for quality control and assurance by an independent senior reviewer (OM) to ensure completeness and correctness.

Data Items

The following categories of data will be abstracted, administrative information (author, year of publication, year of data collection, citation, country/region, funding source), methods (study design, population, sample size, laboratory procedures) and results (malaria symptom resolution, IC_{50}, $RSA_{0\text{-}3hrs}$, TSA_{50}, malaria parasite suppression rate) as illustrated in the PICOST (Table 2)

Risk of bias assessment

Two members of the research team (LN & KOO) shall independently evaluate the methodological quality of included non-randomized studies using a modified version of the Newcastle-Ottawa Scale (NOS) (17). The tool includes seven domains scored from 0 (high risk of bias) to 3 (low risk of bias), the mean of domains shall be considered to result in a score between 0 and 3, where a higher score represents a lower risk of bias. Consensus on any disagreement in the quality assessment shall reached through discussion and consultation with an independent senior reviewer. For randomized controlled trials (RCTs), we shall apply the Cochrane risk of bias tool to assess potential risk of bias(18). Bias is assessed as a judgement (high, low, and unclear) for individual elements from five domains (selection bias, attrition bias, performance bias, reporting bias, detection bias and other biases for example conflict of interest). Any disagreements will be resolved through discussion and further disagreement referred to the tie breaker (MO).

Publication bias

The included articles will be assessed for publication bias using the asymmetry of the funnel plots and/or Egger's test as appropriate (19). These are rank-based data augmentation techniques that have been shown to be accurate for assessing publication bias due to missing data/studies. We will create funnel plots and use the symmetry of the plots to detect the likelihood of publication bias among the articles included in the review. In the absence of missing studies, the scatter plot resembles a symmetrical inverted funnel with a wide base and a narrow top (20). The presence of large "holes" or asymmetry in the plot indicates publication bias but could also be explained by other factors such as study heterogeneity.

Heterogeneity

The I^2-statistic will be used to assess the level of statistical heterogeneity in the articles. The I squared statistic will show the percentage (%) of heterogeneity attributable to between-study variation (21). Heterogeneity will be categorized as, low (I^2=25%) (low), moderate (I^2 =50) and high (I^2 >75%). Sub-group analysis will be done among articles categorized as low and moderate heterogeneity.

Quality assessment

The AMSTAR-2 critical appraisal tool will be used to assess the quality of evidence (22). The tool includes ten domains against which the articles' quality will be assessed. The overall quality of evidence will be assessed using a modified GRADE approach in which we assigned certainty of evidence ratings for the above-mentioned outcome variables using an approach developed by the GRADE Working Group (23) and this will be done in duplicate, with any disagreements resolved by consensus.

Criteria for determination of independent findings

Dependence may occur at the study or intra-study levels. At the study level, the most complete and latest report, where available, will be selected in case of multiple reports of a single study. However, if different reports discuss different sub-groups or outcomes, the data from all these reports will be treated as a single case, using integrative approach (24). At the intra-study level, only a single effect from each study will be included in each meta-analysis. Where studies report multiple effects for different outcome types, these will be synthesized separately. Where studies report multiple dependent effects for a particular outcome type, we shall use 'synthetic effects' to generate a sample-weighted average prior to incorporation in meta-analysis.

Missing data

In case of missing data from the published articles, study authors will be contacted. When the author cannot be accessed or In case of none response from authors, we will report the characteristics of the study but will not include such a study in the meta-analysis. Where studies do not report group sample sizes to calculate the standard error of the standardized mean difference, the following approximation will be used:

$$se(d) = \sqrt{\frac{4}{N} + \frac{d^2}{2N}}$$

where se(d) is the standard error of the standardized mean difference, d is the standardized mean difference and N is the total sample size.

Data synthesis

Standardized mean differences (SMDs) from continuous outcome variables and odds ratios or prevalence ratios for dichotomous outcome variables will be synthesized separately. Effect sizes will be pooled statistically using inverse variance weighted random effects meta-analysis, using the mean command in Stata v16. Pooled effects will be expressed in metric that is relevant, for example, a percentage change in odds, or a mean difference measured in natural units of outcome.

The synthesis will further be in form of summary of findings tables, simple graphs and forest plots as applicable using a STATA v16. This will follow the format of the Cochrane consumers and communication review group (25). We shall describe the included articles, group articles according to study design and type of intervention, organize and tabulate results to identify patterns and transform the results into a common descriptive format. These will be in form of outcome data tables, simple graphs and forest plots as applicable. These will feed into the summary of findings tables that inform the syntheses for sharing. We shall thus use both narrative and quantitative synthesis.

Sensitivity analysis

The sensitivity analysis will be done by removing studies from the meta- analysis one-by-one to see if the results of the meta-analysis are sensitive to any single study. We will also examine sensitivity of findings to risk of bias status (low risk, some concerns and high risk).

Ethics and dissemination

The review protocol was reviewed and approved by the School of Biomedical Science Research Ethics Committee, Makerere University College of Health Sciences (SBS-2022-213). Results will be disseminated through conference presentations and publication in a peer-reviewed journal.

Patient and public involvement

Patients or the public will not be involved in the design, or conduct, or reporting, or dissemination plans of our research.

DISCUSSION

The accessibility to quality and efficacious antimalarial medicines is fundamental towards successful malaria treatment. However, this may be compromised by the inaccessibility and high cost of these antimalarials in communities in addition to the rising rate of resistance. There is therefore need to accelerate research in discovery and development of novel efficacious and less toxic antimalarials.

Herbal extracts have proven to contain various phytochemicals constitutes which have pharmacological properties. Globally, researchers have carried out primary studies that have documented and provided knowledge on the anti-plasmodial activities of numerous plants. This systematic review and evidence and gap map will therefore collate and map the available evidence, identify the gaps, and synthesize the efficacy of herbal antimalarial medicines in malaria affected regions globally in comparison with the recommended artemisinin on market. This will then inform on the most efficacious herbal extract that can be used for the drug development process.

Abbreviations

IC_{50}, Half-maximal inhibitory concentration

$RSA_{0\text{-}3h}$, Ring-stage survival assay

$TSA_{50,}$ Trophozoite Survival Assay

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Author contributions

Conceptualization of the study was done by (MO, NL, KOO). MO, NL and KOO drafted the protocol, Critical review (MO, NL, KOO, AAK, EAO), and approval of the final version (MO, NL, KOO, AAK, EAO).

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Conflict of interest

The authors have no conflict of interest to declare.

Competing interests

None declared.

Patient consent for publication

Not applicable

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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Reported on Page # (Line Number)
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	**1(1)**
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	**NA**
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	**1(23-24)**
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	**9 (382-395)**
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	**9(397-400)**
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	**NA**
Support:			
Sources	5a	Indicate sources of financial or other support for the review	**10(403-406)**
Sponsor	5b	Provide name for the review funder and/or sponsor	**10(403-406)**
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	**10(403-406)**
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	**2(82-99)**
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	**4-5(160-166)**
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	**5(179-192)**
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	**6(197-208)**
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	**6-7(210-257)**

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	**7(250-257)**
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	**7(250-257)**
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	**7(259-269)**
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	**7(266-271)**
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	**5(168-176)**
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	**7(271-283)**
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	**8(332-346)**
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	**7(295-300)**
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	**8(348-351)**
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	**7(285-293)**
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	**8(303-309)**

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

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EFFICACY OF ANTI-MALARIAL HERBAL MEDICINES USED BY COMMUNITIES IN MALARIA AFFECTED REGIONS GLOBALLY: A PROTOCOL FOR SYSTEMATIC REVIEW AND EVIDENCE AND GAP MAP

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EFFICACY OF ANTI-MALARIAL HERBAL MEDICINES USED BY COMMUNITIES IN MALARIA AFFECTED REGIONS GLOBALLY: A PROTOCOL FOR SYSTEMATIC REVIEW AND EVIDENCE AND GAP MAP

Authors

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ABSTRACT

Introduction: With the rising resistance to Artemisinin Combination Therapies (ACTs), there is a need to hasten the discovery and development of newer anti-malarial agents. Herbal medicines are key for the development of novel drugs. Currently, herbal medicine usage in communities for treatment of malaria symptoms is common as an alternative to conventional (modern) anti-malarial agents. However, the efficacy and safety of most of the herbal medicines has not yet been established. Therefore, this systematic review and evidence and gap map is intended to collate and map the available evidence, identify the gaps, and synthesize the efficacy of herbal antimalarial medicines used in malaria affected regions globally.

Methods and analysis: The systematic review and evidence and gap map (EGM) will be done following PRISMA and Campbell Collaboration guidelines respectively. This protocol has been registered in PROSPERO. Data sources will include PubMed, MEDLINE Ovid, EMBASE, Web of Science, Google Scholar, and grey literature search. Data extraction will be done in duplicate using a data extraction tool tailored in Microsoft Office excel for herbal antimalarials discovery research questions following the PICOST framework. The Risk of Bias and overall quality of evidence will be assessed using Cochrane risk of bias tool (clinical trials), QUIN tool (*In-vitro* studies*)*, Newcastle Ottawa tool (Observational studies) and SYRCLE's risk of bias tool for animal studies (*In-vivo* studies). Data analysis will be done using both structured narrative and quantitative synthesis. The primary review outcomes will be clinically important efficacy and adverse drug reactions. Laboratory parameters will include IC_{50}, $RSA_{0\text{-}3h}$, TSA_{50}, and Adequate Clinical and Parasitological response.

Ethics and dissemination: The review protocol was approved by the School of Biomedical Science Research Ethics Committee, Makerere University College of Health Sciences (SBS-2022-213).

PROSPERO Registration Number: CRD42022367073

Key words: Herbal medicines, Antimalarial, Plasmodium falciparum, malaria, herbal extracts

Article summary

Strengths and limitations of this study

Strength

- The review will provide an evidence gap map on use of herbal antimalarial agents for malaria management in communities affected by malaria globally.
- The review will provide an update on efficacy of herbal products against malaria parasites.
- The study will combine both evidence synthesis and evidence gap map on herbal antimalarial products in malaria affected regions.

Limitations

- The review will focus only on herbal medicines whose efficacy has been compared against artemisinin agents as these are the current cornerstone in malaria treatment
- The review will focus on herbal antimalarial medicines in malaria affected regions only.

INTRODUCTION

Malaria remains a global public health problem affecting nearly half of the world's population. In the year 2020, global estimates indicated 241 million malaria cases and 627 000 malaria deaths (1). Malaria was still deadly in 2021 as the 6th most important cause of death in Africa ahead of COVID-19 that was the 7th, up from the 22nd in 2020 driven by the *delta* variant. Sub-Saharan Africa carries the greatest burden, accounting for about 95% of all malaria cases and 96% of all deaths in 2020 (1).

Chemotherapy with effective antimalarial medicines remains the most predominant intervention for effective management of malaria globally (2). Artemisinin-based combination therapies (ACTs) are the main stay and frontline treatments for uncomplicated *Plasmodium falciparum* malaria (3). However, malaria treatment faces a number of challenges globally including; drug resistance, poor quality medicines, inaccessibility, unavailability and high cost (4). Resistance to ACTs was originally reported in areas of the Greater Mekong Sub region (GMS) such as Thailand and Cambodia (5). Currently, artemisinin resistance has also independently arisen in East Africa (Rwanda and Uganda) (5). This is a major threat for the global initiative on elimination and eradication of malaria (6) justifying concerted efforts to hasten the discovery and development of newer anti-malarial molecules.

Traditional medicines (herbs) remain a corner stone for the discovery of novel drugs with more desirable medicinal and pharmacological properties (7, 8). Use of traditional medicine for treatment of malaria symptoms has previously been achieved with *Quinghao* isolated from a Chinese herbal medicine, *Artemisia annua* and quinine isolated from *Cinchona* species (Rubiaceae) (9, 10). In various malaria affected regions globally, plants are traditionally used to alleviate symptoms of malaria such as fevers and treat malaria. This is due to inherited cultural practices and belief in traditional medicine, accessibility and relatively lower costs compared to the modern medicines, largely unknown and underappreciated toxicities and perceived efficacy (11).

Why this review?

Communities have now resorted to use of natural plant products (herbs) that are assumed or claimed to have antimalarial efficacy. With the prevailing challenges facing the use of ACTs, use of herbal products and development of alternative conventional antimalarial medicines is key for malaria control and eradication efforts globally. However, the antimalarial efficacy of most herbal agents used by communities in malaria affected regions remains unknown. The continued use of herbal agents with unproven efficacy is potentially harmful to the population as it can lead to unwanted outcomes such as delay in getting effective treatment, morbidity, and mortality. There is thus need, to establish the comparative antimalarial efficacy of herbal medicines and

artemisinin agents used in malaria affected regions globally. This will help guide the current efforts to develop alternative antimalarial agents globally.

Several reviews have been conducted documenting information on different plants used for their antimalarial activities. (11-13). However, these reviews focused on studies that investigated *In-vitro* and *In-vivo* activities of these herbs without comparing their efficacies with the already existing modern anti-malarial medicines. Our current review seeks to collate and map evidence comparing the antimalarial efficacy of herbal medicines with that of artemisinin agents in malaria affected regions globally.

How the intervention might work

Early detection and treatment remain the principal strategies for control of malaria globally. Treatment of malaria currently involves the use of artemisinin combination therapies as recommended by WHO globally. However, this strategy is affected by the rising levels of resistance to artemisinin and the partner drugs and the relatively high cost. Artemisinin agents are the current cornerstone in treatment of malaria globally however, they are faced with the threat of resistance development. Herbal medicines with anti-plasmodial activity are used by communities in treatment of malaria in malaria affected regions globally. They are therefore used as alternatives to artemisinin agents in malaria affected regions globally especially in areas with reported partial resistance to artemisinin agents. Efficacious herbal medicines could potentially provide activity against both sensitive and slow artemisinin clearing parasites. Herbal antimalarial products may also help improve access to malaria treatment due to the cheaper cost, and convenient dosing. Thus, potentially contributing to the anti-malarial drug development pipeline to help provide alternative to the current artemisinin agents.

METHODS

This will be a multi-modal blended systematic review and evidence gap map. The evidence and gap map and systematic review will be done following the Campbell Collaboration (14) and Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines respectively (15). There will be no language restriction.

This protocol was registered in PROPSERO (https://www.crd.york.ac.uk/prospero/) registration number CRD42022367073 (16)

Intervention-outcome framework for the evidence and gap map

The intervention domains will include medicinal plants and plant extracts. There will be two outcome domains clinical (symptom resolution; fever, body aches/fatigue, malaise, joint pains, nausea/vomiting, loss of appetite, Adequate clinical and parasitological response) and laboratory (IC_{50}, $RSA_{0\text{-}3hrs}$, TSA_{50}, parasite clearance rate) (Table 1).

Table 1: Intervention-outcome domains for the evidence and gap map

Intervention Domains	***Indicators***	***Description***
• *Medicinal plants*	• Kind/type of plant (shrub, herb, tree)	• The type of plant, including geographical location
	• Parts of the plant used (leaves, back, roots, shoots)	• Plant material used, either entire plant or its parts (single or combined)
	• Name of the plant species	• Include both Genus and species names
	• Age of the plant/part	• Either young or mature/old plant/or parts
• *Plant extract*	• Water extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Methanol extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Ether extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Chloroform extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Ethanolic extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Crude extract	• Proportions of the different extracts in the mixture
Outcomes Domains	Indicators	Description
• *Clinical outcomes*	• Fever	• Relief from fever
	• Body aches/malaise	• Relief from body aches
	• Joint pains	• Relief from joint pains
	• Nausea/vomiting	• Relief from vomiting
	• Appetite	• Gaining of appetite
	• Adequate clinical and	• Resolution of clinical

	parasitological response	symptoms of malaria and Plasmodium parasite clearance
• *Laboratory outcomes*	• IC_{50},	• Inhibitory concentration killing 50% of parasites
	• $RSA_{0\text{-}3hrs}$	• Concentration killing early-stage parasites (0-3hrs)
	• TSA_{50}	• Concentration killing 50% of trophozoites

Review question

What is the clinical, *In-vitro* and *In-vivo* efficacy of anti-malarial herbal medicines used by communities in malaria affected regions globally?

Table 2: PICOST model for the review question

Element of PICO	Description
Population (P)	Clinical: Malaria patients (children and adults) I*n-vitro* studies: Malaria parasites, *Plasmodium spp (e.g: P. falciparum)*. Wild or field strain parasites *In-vivo (animal)* studies*:* Malaria parasites, *Plasmodium spp (e.g P. berghei)*
Intervention/Exposure	Clinical: Use of herbal medicines for management of symptoms of malaria among children and adult patients *In-vitro studies:* Plant extracts used against *Plasmodium* parasites *In-vivo studies:* Plant extracts used against *Plasmodium* parasite infections of laboratory animals
Comparator	• *In-vitro studies:* None • *In-vivo (animal) studies:* Artemisinin antimalarial agents • Clinical studies: Artemisinin based antimalarial agents • For *in-vitro* studies, will use historical data of the IC50 values for Artemisinin agents to compare with the IC50 values obtained from herbal anti-plasmodial assays
Outcomes	Prevalence of use of herbal medicines in treatment of malaria symptoms Clinical: symptom resolution (fever, nausea/vomiting, joint pains, fatigue/malaise, loss of appetite), Adequate clinical and parasitological response (ACPR) Laboratory: IC_{50}, $RSA_{0\text{-}3h}$, TSA_{50}, parasite suppression rate, Plasmodium *spp* clearance, *Plasmodium falciparum* clearance
Setting	Malaria affected countries globally (tropical countries)

	Designs: Cross-sectional studies, Randomized controlled trials (RCTs)
Time	2000 to date (Introduction of Artemisinin in malaria treatment outside China)

Outcomes

Primary outcomes

- Clinical: *Plasmodium falciparum* parasite clearance (Adequate clinical and parasitological response, ACPR), symptom resolution (fever, nausea/vomiting, joint pains, fatigue/malaise, loss of appetite)

- *In-vitro* studies: IC_{50}, $RSA_{0\text{-}3h}$, TSA_{50}

- *In-vivo* (animal) studies: _Parasite suppression rate, Plasmodium *spp* clearance

Secondary outcome

- Rate of symptom resolution (in-vivo and clinical studies)
- Safety of antimalarial herbal medicines; *in-vivo* studies (LD50, organ toxicity, teratogenicity, carcinogenicity, mutagenicity); clinical studies (reported side effects/adverse drug reactions)

Eligibility criteria

Inclusion criteria

- Peer reviewed articles reporting clinical efficacy of herbal medicines in malaria treatment among children and adults in malaria affected regions globally
- Peer reviewed articles reporting on herbal medicinal plants used by communities for management of symptoms of malaria in malaria affected regions globally
- Peer reviewed articles reporting on the safety of herbal medicines used by communities in management of malaria symptoms
- Peer reviewed articles reporting on *in-vitro* efficacy of anti-malarial herbal medicines
- Peer reviewed articles reporting on *in-vivo* efficacy of anti-malarial herbal medicines
- Peer reviewed articles published from the years 2000 to date
- Articles published in both English and non-English language

Exclusion criteria

- Peer reviewed articles whose full text cannot be retrieved. Efforts will be made to retrieve all included full text articles before prior to being excluded. The librarian (AAK) will use external sources like Web of Science, EMBASE, Sci-Hub, Lib-Hub and PDF Drive. In addition, AAK will contact other librarians for retrieval of full text articles. However, full text articles which will still not be able to be accessed despite these efforts will be excluded from the review.
- Peer reviewed articles from studies that did not receive ethical review and approval

Identification of articles

Data Sources

Article search will be performed by an experienced librarian (AAK) in PubMed, MEDLINE Ovid, EMBASE and Web of Science, and other grey literature sources. Medical subject headings (MeSH) terms and Boolean operators “AND” and “OR” will be used during article search. The data sources will include scholarly databases, grey literature sources, contacting authors as well as screening reference sources of included studies. we shall search grey literature from organization websites such as WHO, Medicines for malaria venture, institutional repositories, and contact experts/researchers in malaria field. We will also search Google Scholar for

additional studies that may be missed from the other sources. The search will be limited to 2000 to 2022.

We will also screen through reference lists of included studies for additional eligible studies that may not be identified by the search.

Search Strategy

Full article search has not been done yet, however scoping literature search was completed on 17th November 2022. The search terms below will be used in full article search to identify eligible articles, based on PICOST. Terms relating to the same element of PICOST will be combined using Boolean operator 'OR', while the different concepts / PICO categories will be combined using 'AND'.

Population:

Laboratory: Plasmodium falciparum, Plasmodium species, wild type plasmodium parasites, field plasmodium parasites, laboratory animals (Wister albino rats, Mice).

Clinical: Children, infants, adults

Disease/condition: Malaria, fever, malaria fever, malaria symptomatic patients, Plasmodium falciparum infection, Plasmodium infection

Intervention: Herbal medicine* OR herbal remed* OR medicinal plant* OR herbal formulation* OR plant medicine* OR herbal product* OR plant extract* OR medicinal herb* OR ethnomedicine* OR traditional herbal medicine OR alternative medicine OR ethnobotany OR phyto-medicine OR shrub OR herb OR shoot OR leaves OR roots OR herbal extract

Comparator:

Clinical: Artemisinin based antimalarial agents

Laboratory: Dihydroartemisinin, Lumefantrine, Piperaquine

Outcome:

Clinical: Fever, nausea, vomiting, joint pains, malaise, loss of appetite, symptom resolution, symptom clearance, Adequate clinical and parasitological response

Laboratory: IC_{50}, $RSA_{0\text{-}3hrs}$, TSA_{50}, malaria parasite clearance, Plasmodium *spp* parasite clearance, *Plasmodium falciparum* clearance, malaria parasite suppression rate

Setting: Globally

Time: 2000-2022, in line with the introduction of Artemisinin in malaria treatment.

Evidence and Gap Map (EGM). This will be a secondary product in addition to the systematic review. Our approach to the Evidence Gap Map (EGM) will be informed by the Campbell Collaboration approach (17). An EGM highlights where the evidence is and where more evidence is needed in terms of interventions and outcomes contained in the studies identified for this systematic review. They consolidate what is known and what is not known by mapping out existing and ongoing studies and providing a graphical representation of areas with strong, weak, or no evidence on the effect of interventions.

Briefly, we will apply the data already identified, screened and coded from this systematic review to develop the EGM. Using the EPPI mapper adds-on for EPPIR Web software for conducting systematic reviews we will produce an EGM in visual presentation of the evidence matrix. The intervention categories lie on the y-axis whilst outcome domains will run in the x-axis. Additional dimensions of the study or intervention characteristics, such as study design, geographical region and country income subgroup status or population sub-group will be applied as filters.

Data management, screening, and selection

For the initial management of references from search results, EndNote software will be used. The articles will be exported to Endnote v20 and duplicates will be removed. The articles will then be screened in duplicate using predetermined eligibility criteria. The screening will be performed independently by the review team pair (KOO and LN) in EPPI-Reviewer v4.13.0.0, using a screening tool developed *a priori* and piloted using 10% of the search yield, any disagreements between the reviewers will be resolved by consensus, and any further disagreements will be referred to the tie breaker (OM).

Data abstraction and coding

The data abstraction tool will be created and piloted using 10% of the eligible studies to ensure it captures all relevant data from included studies and the uploaded on EPPI-Reviewer v4.13.0.0. The coding process will be carried out independently by two research team members (KOO and LN), whose results will be reconciled and disagreements resolved through discussion, and their results will later be validated for quality control and assurance by an independent senior reviewer (OM) to ensure completeness and correctness.

Data Items

The following categories of data will be abstracted, administrative information (author, year of publication, year of data collection, citation, country/region, funding source), methods (study design, population, sample size, laboratory procedures) and results (malaria symptom resolution, IC_{50}, $RSA_{0\text{-}3hrs}$, TSA_{50}, malaria parasite suppression rate) as illustrated in the PICOST (Table 2)

Risk of bias assessment

Two members of the research team (LN & KOO) shall independently evaluate the methodological quality of included studies. The risk of bias in observational (non-randomized) studies will be assessed using a modified Newcastle-Ottawa Scale (NOS) tool (18). The tool includes seven domains scored from 0 (high risk of bias) to 3 (low risk of bias), the mean of domains shall be considered to result in a score between 0 and 3, where a higher score represents a lower risk of bias. Randomized controlled trials (RCTs), the Cochrane risk of bias tool will be used to assess risk of bias (selection bias, attrition bias, performance bias, reporting bias, detection bias and other biases for example conflict of interest) (19). For *in-vitro* studies the risk of bias will be assessed using QUIN tool (20). The tool has twelve-item criteria which will be scored, and the scores used to grade the *in-vitro* study as high (<50%), medium (50% to 70%), or low (>70%) risk of bias. For *in-vivo* studies, risk of bias will be assessed following SYRCLE's risk of bias tool for animal studies (21). The following risk of bias will be assessed in the *in-vivo* studies, selection bias (sequence generation, baseline characteristics, allocation concealment), performance bias (random housing, blinding), detection bias (random outcome assessment, blinding), attrition bias (incomplete outcome data), reporting bias (selective outcome reporting). The *in-vivo* studies will be scored and assigned a judgement of low, high, or unclear risk of bias. Consensus on any disagreement in the quality assessment will be reached through discussion and consensus between the two independent reviewers (LN, KOO). Any further disagreement will be resolved through a tiebreaker (MO). For the EGM, the AMSTAR-2 tool will only be used for assessing risk of bias of the included systematic review articles (22).

Publication bias

The included articles will be assessed for publication bias using the asymmetry of the funnel plots and/or Egger's test as appropriate (23). These are rank-based data augmentation techniques that have been shown to be accurate for assessing publication bias due to missing data/studies. We will create funnel plots and use the symmetry of the plots to detect the likelihood of publication bias among the articles included in the review. In the absence of missing studies, the scatter plot resembles a symmetrical inverted funnel with a wide base and a narrow top (24). The presence of large "holes" or asymmetry in the plot indicates publication bias but could also be explained by other factors such as study heterogeneity. The performance of Egger's tests has been extensively studied for binary outcomes, but not for continuous ones. In this study we shall

use Egger's test for binary outcomes. For continuous outcomes, we shall assess baseline risk of bias in the included studies and assess publication bias using standard errors (25)

Assessment of strength and confidence of cummulative evidence

The overall strength of evidence will be assessed using a modified GRADE approach in which we assigned certainty of evidence ratings for the above-mentioned outcome variables using an approach developed by the GRADE Working Group (26) and this will be done in duplicate, with any disagreements resolved by consensus.

Heterogeneity

The I^2-statistic will be used to assess the level of statistical heterogeneity in the articles. The I squared statistic will show the percentage (%) of heterogeneity attributable to between-study variation (27). Heterogeneity will be categorized as, low (I^2=25%) (low), moderate (I^2 =50) and high (I^2 >75%). Sub-group analysis will be done among articles categorized as low and moderate heterogeneity.

Criteria for determination of independent findings

Dependence may occur at the study or intra-study levels. At the study level, the most complete and latest report, where available, will be selected in case of multiple reports of a single study. However, if different reports discuss different sub-groups or outcomes, the data from all these reports will be treated as a single case, using integrative approach (28). At the intra-study level, only a single effect from each study will be included in each meta-analysis. Where studies report multiple effects for different outcome types, these will be synthesized separately. Where studies report multiple dependent effects for a particular outcome type, we shall use 'synthetic effects' to generate a sample-weighted average prior to incorporation in meta-analysis.

Missing data

In case of missing data from the published articles, study authors will be contacted. When the author cannot be accessed or in case of no response from authors, we will report the characteristics of the study but will not include such a study in the meta-analysis. Where studies do not report group sample sizes to calculate the standard error of the standardized mean difference, the following approximation will be used:

$$se(d) = \sqrt{\frac{4}{N} + \frac{d^2}{2N}}$$

where se(d) is the standard error of the standardized mean difference, d is the standardized mean difference and N is the total sample size (29)

Data synthesis

Standardized mean differences (SMDs) from continuous outcome variables and odds ratios or prevalence ratios for dichotomous outcome variables will be synthesized separately. Effect sizes will be pooled statistically using inverse variance weighted random effects meta-analysis, using the metan command in Stata v16. Pooled effects will be expressed in metric that is relevant, for example, a percentage change in odds, or a mean difference measured in natural units of outcome.

The synthesis will further be in form of summary of findings tables, simple graphs and forest plots as applicable using a STATA v16. This will follow the format of the Cochrane consumers and communication review group (30). We shall describe the included articles, group articles according to study design and type of intervention, organize and tabulate results to identify patterns and transform the results into a common descriptive format. These will be in form of outcome data tables, simple graphs and forest plots as applicable. These will feed into the summary of findings tables that inform the syntheses for sharing. We shall thus use both narrative and quantitative synthesis.

Sensitivity analysis

The sensitivity analysis will be done by removing studies from the meta- analysis one-by-one to see if the results of the meta-analysis are sensitive to any single study. We will also examine sensitivity of findings to risk of bias status (low risk, some concerns and high risk).

Ethics and dissemination

The review protocol was reviewed and approved by the School of Biomedical Science Research Ethics Committee, Makerere University College of Health Sciences (SBS-2022-213). The protocol was further cleared by Uganda National Council of Science and Technology, UNCST. Results will be disseminated through conference presentations and publication in peer-reviewed journal.

Patient and public involvement

Patients or the public will not be involved in the design, or conduct, or reporting, or dissemination plans of our research.

DISCUSSION

The accessibility to quality and efficacious antimalarial medicines is fundamental towards successful malaria treatment. However, this may be compromised by the inaccessibility and high cost of these antimalarials in communities in addition to the rising rate of resistance. There is therefore need to accelerate research in discovery and development of novel efficacious and less toxic antimalarials.

Herbal extracts have proven to contain various phytochemicals which have pharmacological properties. Globally, researchers have carried out primary studies that have documented and provided knowledge on the anti-plasmodial activities of numerous plants. This systematic review and evidence and gap map will therefore collate and map the available evidence, identify the gaps, and synthesize the efficacy of herbal antimalarial medicines in malaria affected regions globally in comparison with the recommended artemisinin on market. This will help collate evidence on the most efficacious herbal extract that can be used to inform the antimalarial drug development process.

Abbreviations

IC_{50}, Half-maximal inhibitory concentration

$RSA_{0\text{-}3h}$, Ring-stage survival assay

$TSA_{50,}$ Trophozoite Survival Assay

Author contributions

Conceptualization of the study was done by (MO, NL, KOO). MO, NL and KOO drafted the protocol, Critical review (MO, NL, KOO, AAK, RA, EAO), and approval of the final version (MO, NL, KOO, AAK, RA, EAO).

Funding

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Conflict of interest

The authors have no conflict of interest to declare.

Competing interests

None declared.

Patient consent for publication

Not applicable

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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Reported on Page # (Line Number)
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	**1(1)**
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	**NA**
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	**1(38); 3(121-122)**
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	**10 (417-430); 1(9-12)**
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	**10(432-435)**
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	**NA**
Support:			
Sources	5a	Indicate sources of financial or other support for the review	**10(438-441)**
Sponsor	5b	Provide name for the review funder and/or sponsor	**10(438-441)**
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	**10(438-441)**
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	**2(83-93)**
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	**4-5(165-173)**
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	**6(191-211)**
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	**6(213-225)**
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	**6-7(227-261)**

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	**7(279-286)**
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	**7(279-286)**
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	**7(289-295)**
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	**8(287-301)**
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	**5-6(175-189)**
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	**8(303-323)**
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	**9(371-385)**
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	**8(344-349)**
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	**9(388-391)**
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	**8(325-336)**
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	**8(338-342)**

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

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EFFICACY OF ANTI-MALARIAL HERBAL MEDICINES USED BY COMMUNITIES IN MALARIA AFFECTED REGIONS GLOBALLY: A PROTOCOL FOR SYSTEMATIC REVIEW AND EVIDENCE AND GAP MAP

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EFFICACY OF ANTI-MALARIAL HERBAL MEDICINES USED BY COMMUNITIES IN MALARIA AFFECTED REGIONS GLOBALLY: A PROTOCOL FOR SYSTEMATIC REVIEW AND EVIDENCE AND GAP MAP

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ABSTRACT

Introduction: With the rising resistance to Artemisinin-based combination treatment (ACTs), there is a need to hasten the discovery and development of newer anti-malarial agents. Herbal medicines are key for the development of novel drugs. Currently, herbal medicine usage in communities for treatment of malaria symptoms is common as an alternative to conventional (modern) anti-malarial agents. However, the efficacy and safety of most of the herbal medicines has not yet been established. Therefore, this systematic review and evidence and gap map is intended to collate and map the available evidence, identify the gaps, and synthesize the efficacy of herbal antimalarial medicines used in malaria affected regions globally.

Methods and analysis: The systematic review and evidence and gap map (EGM) will be done following PRISMA and Campbell Collaboration guidelines respectively. This protocol has been registered in PROSPERO. Data sources will include PubMed, MEDLINE Ovid, EMBASE, Web of Science, Google Scholar, and grey literature search. Data extraction will be done in duplicate using a data extraction tool tailored in Microsoft Office excel for herbal antimalarials discovery research questions following the PICOST framework. The Risk of Bias and overall quality of evidence will be assessed using Cochrane risk of bias tool (clinical trials), QUIN tool (*In-vitro* studies*)*, Newcastle Ottawa tool (Observational studies) and SYRCLE's risk of bias tool for animal studies (*In-vivo* studies). Data analysis will be done using both structured narrative and quantitative synthesis. The primary review outcomes will be clinically important efficacy and adverse drug reactions. Laboratory parameters will include IC_{50}, $RSA_{0\text{-}3h}$, TSA_{50}.

Ethics and dissemination: The review protocol was approved by the School of Biomedical Science Research Ethics Committee, Makerere University College of Health Sciences (SBS-2022-213).

PROSPERO Registration Number: CRD42022367073

Key words: Herbal medicines, Antimalarial, Plasmodium falciparum, malaria, herbal extracts

Article summary

Strengths and limitations of this study

Strength

- The review will provide an evidence gap map on use of herbal antimalarial agents for malaria management in communities affected by malaria globally.
- The review will provide an update on efficacy of herbal products against malaria parasites.
- The study will combine both evidence synthesis and evidence gap map on herbal antimalarial products in malaria affected regions.

Limitations

- The review will focus only on herbal medicines whose efficacy has been compared against artemisinin agents as these are the current cornerstone in malaria treatment
- The review will focus on herbal antimalarial medicines in malaria affected regions only.

INTRODUCTION

Malaria remains a global public health problem affecting nearly half of the world's population. In the year 2020, global estimates indicated 241 million malaria cases and 627 000 malaria deaths (1). Malaria was still deadly in 2021 as the 6th most important cause of death in Africa ahead of COVID-19 that was the 7th, up from the 22nd in 2020 driven by the *delta* variant. Sub-Saharan Africa carries the greatest burden, accounting for about 95% of all malaria cases and 96% of all deaths in 2020 (1).

Chemotherapy with effective antimalarial medicines remains the most predominant intervention for effective management of malaria globally (2). Artemisinin-based combination therapies (ACTs) are the main stay and frontline treatments for uncomplicated *Plasmodium falciparum* malaria (3). However, malaria treatment faces a number of challenges globally including; drug resistance, poor quality medicines, inaccessibility, unavailability and high cost (4). Resistance to ACTs was originally reported in areas of the Greater Mekong Sub region (GMS) such as Thailand and Cambodia (5). Currently, artemisinin resistance has also independently arisen in East Africa (Rwanda and Uganda) (5). This is a major threat for the global initiative on elimination and eradication of malaria (6) justifying concerted efforts to hasten the discovery and development of newer anti-malarial molecules.

Traditional medicines (herbs) remain a corner stone for the discovery of novel drugs with more desirable medicinal and pharmacological properties (7, 8). Use of traditional medicine for treatment of malaria symptoms has previously been achieved with *Quinghao* isolated from a Chinese herbal medicine, *Artemisia annua* and quinine isolated from *Cinchona* species (Rubiaceae) (9, 10). In various malaria affected regions globally, plants are traditionally used to alleviate symptoms of malaria such as fevers and treat malaria. This is due to inherited cultural practices and belief in traditional medicine, accessibility and relatively lower costs compared to the modern medicines, largely unknown and underappreciated toxicities and perceived efficacy (11).

Why this review?

Communities have now resorted to use of natural plant products (herbs) that are assumed or claimed to have antimalarial efficacy. With the prevailing challenges facing the use of ACTs, use of herbal products and development of alternative conventional antimalarial medicines is key for malaria control and eradication efforts globally. However, the antimalarial efficacy of most herbal agents used by communities in malaria affected regions remains unknown. The continued use of herbal agents with unproven efficacy is potentially harmful to the population as it can lead to unwanted outcomes such as delay in getting effective treatment, morbidity, and mortality. There is thus need, to establish the comparative antimalarial efficacy of herbal medicines and artemisinin agents used in malaria affected regions globally. This will help guide the current efforts to develop alternative antimalarial agents globally.

Several reviews have been conducted documenting information on different plants used for their antimalarial activities. (11-13). However, these reviews focused on studies that investigated *In-vitro* and *In-vivo* activities of these herbs without comparing their efficacies with the already existing modern anti-malarial medicines. Our current review seeks to collate and map evidence comparing the antimalarial efficacy of herbal medicines with that of artemisinin agents in malaria affected regions globally.

How the intervention might work

Early detection and treatment remain the principal strategies for control of malaria globally. Treatment of malaria currently involves the use of artemisinin-based combination treatment as recommended by WHO globally. However, this strategy is affected by the rising levels of resistance to artemisinin and the partner drugs and the relatively high cost. Artemisinin agents are the current cornerstone in treatment of malaria globally however, they are faced with the threat of resistance development. Herbal medicines with anti-plasmodial activity are used by communities in treatment of malaria in malaria affected regions globally. They are therefore used as alternatives to artemisinin agents in malaria affected regions globally especially in areas with reported partial resistance to artemisinin agents. Efficacious herbal medicines could potentially provide activity against both sensitive and slow artemisinin clearing parasites. Herbal antimalarial products may also help improve access to malaria treatment due to the cheaper cost, and convenient dosing. Thus, potentially contributing to the anti-malarial drug development pipeline to help provide alternative to the current artemisinin agents.

METHODS

This will be a multi-modal blended systematic review and evidence gap map. The evidence and gap map and systematic review will be done following the Campbell Collaboration (14) and Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines respectively (15). There will be no language restriction.

This protocol was registered in PROPSERO (https://www.crd.york.ac.uk/prospero/) registration number CRD42022367073 (16)

Intervention-outcome framework for the evidence and gap map

The intervention domains will include medicinal plants and plant extracts. There will be two outcome domains clinical (symptom resolution; fever, body aches/fatigue, malaise, joint pains, nausea/vomiting, loss of appetite, Adequate clinical and parasitological response) and laboratory (IC_{50}, $RSA_{0\text{-}3hrs}$, TSA_{50}, parasite clearance rate) (Table 1).

Table 1: Intervention-outcome domains for the evidence and gap map

Intervention Domains	*Indicators*	*Description*
• *Medicinal plants*	• Kind/type of plant (shrub, herb, tree)	• The type of plant, including geographical location
	• Parts of the plant used (leaves, back, roots, shoots)	• Plant material used, either entire plant or its parts (single or combined)
	• Name of the plant species	• Include both Genus and species names
	• Age of the plant/part	• Either young or mature/old plant/or parts
• *Plant extract*	• Water extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Methanol extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Ether extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Chloroform extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Ethanolic extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
	• Crude extract	• Proportions of the different extracts in the mixture
Outcomes Domains	Indicators	Description
• *Clinical outcomes*	• Fever	• Relief from fever
	• Body aches/malaise	• Relief from body aches
	• Joint pains	• Relief from joint pains
	• Nausea/vomiting	• Relief from vomiting
	• Appetite	• Gaining of appetite
	• Adequate clinical and parasitological response (17)	• Resolution of clinical symptoms of malaria and Plasmodium parasite clearance

• *Laboratory outcomes*	• IC_{50},	• Inhibitory concentration killing 50% of parasites
	• RSA_{0-3hrs}	• Concentration killing early-stage parasites (0-3hrs)
	• TSA_{50}	• Concentration killing 50% of trophozoites

Review question

What is the clinical, *In-vitro* and *In-vivo* efficacy of anti-malarial herbal medicines used by communities in malaria affected regions globally?

Table 2: PICOST model for the review question

Element of PICO	Description
Population (P)	Clinical: Malaria patients (children and adults) *In-vitro* studies: Malaria parasites, *Plasmodium spp (e.g: P. falciparum)*. Wild or field strain parasites *In-vivo (animal)* studies*:* Malaria parasites, *Plasmodium spp (e.g P. berghei)*
Intervention/Exposure	Clinical: Use of herbal medicines for management of symptoms of malaria among children and adult patients *In-vitro studies:* Plant extracts used against *Plasmodium* parasites *In-vivo studies:* Plant extracts used against *Plasmodium* parasite infections of laboratory animals
Comparator	• *In-vitro studies:* None • *In-vivo (animal) studies:* Artemisinin antimalarial agents • Clinical studies: Artemisinin based antimalarial agents • For *in-vitro* studies, will use historical data of the IC50 values for Artemisinin agents to compare with the IC50 values obtained from herbal anti-plasmodial assays
Outcomes	Prevalence of use of herbal medicines in treatment of malaria symptoms Clinical: symptom resolution (fever, nausea/vomiting, joint pains, fatigue/malaise, loss of appetite), Adequate clinical and parasitological response (ACPR) Laboratory: IC_{50}, RSA_{0-3h}, TSA_{50}, parasite suppression rate, Plasmodium *spp* clearance, *Plasmodium falciparum* clearance
Setting	Malaria affected countries globally (tropical countries) Designs: Cross-sectional studies, Randomized controlled trials (RCTs)
Time	2000 to date (Introduction of Artemisinin in malaria treatment

	outside China)

Outcomes

Primary outcomes

- Clinical: *Plasmodium falciparum* parasite clearance (Adequate clinical and parasitological response, ACPR), symptom resolution (fever, nausea/vomiting, joint pains, fatigue/malaise, loss of appetite)

- *In-vitro* studies: IC_{50}, $RSA_{0\text{-}3h}$, TSA_{50}

- *In-vivo* (animal) studies: _Parasite suppression rate, Plasmodium *spp* clearance

Secondary outcome

- Safety of antimalarial herbal medicines; *in-vivo* studies (LD50, organ toxicity, teratogenicity, carcinogenicity, mutagenicity); clinical studies (reported side effects/adverse drug reactions)

Eligibility criteria

Inclusion criteria

- Peer reviewed articles reporting clinical efficacy of herbal medicines in malaria treatment among children and adults in malaria affected regions globally
- Peer reviewed articles reporting on herbal medicinal plants used by communities for management of symptoms of malaria in malaria affected regions globally
- Peer reviewed articles reporting on the safety of herbal medicines used by communities in management of malaria symptoms
- Peer reviewed articles reporting on *in-vitro* efficacy of anti-malarial herbal medicines
- Peer reviewed articles reporting on *in-vivo* efficacy of anti-malarial herbal medicines
- Peer reviewed articles published from the years 2000 to date
- Articles published in both English and non-English language

Exclusion criteria

- Peer reviewed articles whose full text cannot be retrieved. Efforts will be made to retrieve all included full text articles before prior to being excluded. The librarian (AAK) will use external sources like Web of Science, EMBASE, Sci-Hub, Lib-Hub and PDF Drive. In addition, AAK will contact other librarians for retrieval of full text articles. However, full text articles which will still not be able to be accessed despite these efforts will be excluded from the review.
- Peer reviewed articles from studies that did not receive ethical review and approval

Identification of articles

Data Sources

Article search will be performed by an experienced librarian (AAK) in PubMed, MEDLINE Ovid, EMBASE and Web of Science, and other grey literature sources. Medical subject headings (MeSH) terms and Boolean operators “AND” and “OR” will be used during article search. The data sources will include scholarly databases, grey literature sources, contacting authors as well as screening reference sources of included studies. we shall search grey literature from organization websites such as WHO, Medicines for malaria venture, institutional repositories, and contact experts/researchers in malaria field. We will also search Google Scholar for additional studies that may be missed from the other sources. The search will be limited to 2000 to 2022.

We will also screen through reference lists of included studies for additional eligible studies that may not be identified by the search.

Search Strategy

Full article search has not been done yet, however scoping literature search was completed on 17th November 2022. The search terms below will be used in full article search to identify eligible articles, based on PICOST. Terms relating to the same element of PICOST will be combined using Boolean operator 'OR', while the different concepts / PICO categories will be combined using 'AND'.

Population:

Laboratory: Plasmodium falciparum, Plasmodium species, wild type plasmodium parasites, field plasmodium parasites, laboratory animals (Wister albino rats, Mice).

Clinical: Children, infants, adults

Disease/condition: Malaria, fever, malaria fever, malaria symptomatic patients, Plasmodium falciparum infection, Plasmodium infection

Intervention: Herbal medicine* OR herbal remed* OR medicinal plant* OR herbal formulation* OR plant medicine* OR herbal product* OR plant extract* OR medicinal herb* OR ethnomedicine* OR traditional herbal medicine OR alternative medicine OR ethnobotany OR phyto-medicine OR shrub OR herb OR shoot OR leaves OR roots OR herbal extract

Comparator:

Clinical: Artemisinin based antimalarial agents

Laboratory: Dihydroartemisinin, Lumefantrine, Piperaquine, Amodiaquine, Pyronaridine, Chloroquine, Quinine

Outcome:

Clinical: Fever, nausea, vomiting, joint pains, malaise, loss of appetite, symptom resolution, symptom clearance, Adequate clinical and parasitological response

Laboratory: IC_{50}, $RSA_{0\text{-}3hrs}$, TSA_{50}, malaria parasite clearance, Plasmodium *spp* parasite clearance, *Plasmodium falciparum* clearance, malaria parasite suppression rate

Setting: Globally

Time: 2000-2022, in line with the introduction of Artemisinin in malaria treatment.

Evidence and Gap Map (EGM). This will be a secondary product in addition to the systematic review. Our approach to the Evidence Gap Map (EGM) will be informed by the Campbell Collaboration approach (18). An EGM highlights where the evidence is and where more evidence is needed in terms of interventions and outcomes contained in the studies identified for this systematic review. They consolidate what is known and what is not known by mapping out existing and ongoing studies and providing a graphical representation of areas with strong, weak, or no evidence on the effect of interventions.

Briefly, we will apply the data already identified, screened and coded from this systematic review to develop the EGM. Using the EPPI mapper adds-on for EPPIR Web software for conducting systematic reviews we will produce an EGM in visual presentation of the evidence matrix. The intervention categories lie on the y-axis whilst outcome domains will run in the x-axis. Additional dimensions of the study or intervention characteristics, such as study design, geographical region and country income subgroup status or population sub-group will be applied as filters.

Data management, screening, and selection

For the initial management of references from search results, EndNote software will be used. The articles will be exported to Endnote v20 and duplicates will be removed. The articles will then be

screened in duplicate using predetermined eligibility criteria. The screening will be performed independently by the review team pair (KOO and LN) in EPPI-Reviewer v4.13.0.0, using a screening tool developed *a priori* and piloted using 10% of the search yield, any disagreements between the reviewers will be resolved by consensus, and any further disagreements will be referred to the tie breaker (OM).

Data abstraction and coding

The data abstraction tool will be created and piloted using 10% of the eligible studies to ensure it captures all relevant data from included studies and the uploaded on EPPI-Reviewer v4.13.0.0. The coding process will be carried out independently by two research team members (KOO and LN), whose results will be reconciled and disagreements resolved through discussion, and their results will later be validated for quality control and assurance by an independent senior reviewer (OM) to ensure completeness and correctness.

Data Items

The following categories of data will be abstracted, administrative information (author, year of publication, year of data collection, citation, country/region, funding source), methods (study design, population, sample size, laboratory procedures) and results (malaria symptom resolution, IC_{50}, $RSA_{0\text{-}3hrs}$, TSA_{50}, malaria parasite suppression rate) as illustrated in the PICOST (Table 2)

Risk of bias assessment

Two members of the research team (LN & KOO) shall independently evaluate the methodological quality of included studies. The risk of bias in observational (non-randomized) studies will be assessed using a modified Newcastle-Ottawa Scale (NOS) tool (19). The tool includes seven domains scored from 0 (high risk of bias) to 3 (low risk of bias), the mean of domains shall be considered to result in a score between 0 and 3, where a higher score represents a lower risk of bias. Randomized controlled trials (RCTs), the Cochrane risk of bias tool will be used to assess risk of bias (selection bias, attrition bias, performance bias, reporting bias, detection bias and other biases for example conflict of interest) (20). For *in-vitro* studies the risk of bias will be assessed using QUIN tool (21). The tool has twelve-item criteria which will be scored, and the scores used to grade the *in-vitro* study as high (<50%), medium (50% to 70%), or low (>70%) risk of bias. For *in-vivo* studies, risk of bias will be assessed following SYRCLE's risk of bias tool for animal studies (22). The following risk of bias will be assessed in the *in-vivo* studies, selection bias (sequence generation, baseline characteristics, allocation concealment), performance bias (random housing, blinding), detection bias (random outcome assessment, blinding), attrition bias (incomplete outcome data), reporting bias (selective outcome reporting). The *in-vivo* studies will be scored and assigned a judgement of low, high, or unclear risk of bias. Consensus on any disagreement in the quality assessment will be reached through discussion and consensus between the two independent reviewers (LN, KOO). Any further disagreement will be resolved through a tiebreaker (MO). For the EGM, the AMSTAR-2 tool will only be used for assessing risk of bias of the included systematic review articles (23).

Publication bias

The included articles will be assessed for publication bias using the asymmetry of the funnel plots and/or Egger's test as appropriate (24). These are rank-based data augmentation techniques that have been shown to be accurate for assessing publication bias due to missing data/studies. We will create funnel plots and use the symmetry of the plots to detect the likelihood of publication bias among the articles included in the review. In the absence of missing studies, the scatter plot resembles a symmetrical inverted funnel with a wide base and a narrow top (25). The presence of large "holes" or asymmetry in the plot indicates publication bias but could also be explained by other factors such as study heterogeneity. The performance of Egger's tests has been extensively studied for binary outcomes, but not for continuous ones. In this study we shall use Egger's test for binary outcomes (24). For continuous outcomes, we shall assess publication bias using a modified funnel plot and a test considering meta-regression residuals as outcome instead of mean difference and inverse sample size as the exploratory variable unlike standard error (26)

Assessment of strength and confidence of cumulative evidence

The overall strength of evidence will be assessed using a modified GRADE approach in which we assigned certainty of evidence ratings for the above-mentioned outcome variables using an approach developed by the GRADE Working Group (27) and this will be done in duplicate, with any disagreements resolved by consensus.

Heterogeneity

The I^2-statistic will be used to assess the level of statistical heterogeneity in the articles. The I squared statistic will show the percentage (%) of heterogeneity attributable to between-study variation (28). Heterogeneity will be categorized as, low (I^2=25%) (low), moderate (I^2 =50) and high (I^2 >75%) (29). Sub-group analysis will be done among articles categorized as low and moderate heterogeneity.

Criteria for determination of independent findings

Dependence may occur at the study or intra-study levels. At the study level, the most complete and latest report, where available, will be selected in case of multiple reports of a single study. However, if different reports discuss different sub-groups or outcomes, the data from all these reports will be treated as a single case, using integrative approach (30). At the intra-study level, only a single effect from each study will be included in each meta-analysis. Where studies report multiple effects for different outcome types, these will be synthesized separately. Where studies report multiple dependent effects for a particular outcome type, we shall use 'synthetic effects' to generate a sample-weighted average prior to incorporation in meta-analysis.

Missing data

In case of missing data from the published articles, study authors will be contacted. When the author cannot be accessed or in case of no response from authors, we will report the characteristics of the study but will not include such a study in the meta-analysis. Where studies do not report group sample sizes to calculate the standard error of the standardized mean difference, the following approximation will be used:

$$se(d) = \sqrt{\frac{4}{N} + \frac{d^2}{2N}}$$

where se(d) is the standard error of the standardized mean difference, d is the standardized mean difference and N is the total sample size (31)

Data synthesis

Standardized mean differences (SMDs) from continuous outcome variables and odds ratios or prevalence ratios for dichotomous outcome variables will be synthesized separately. Effect sizes will be pooled statistically using inverse variance weighted random effects meta-analysis, using the metan command in Stata v16. Pooled effects will be expressed in metric that is relevant, for example, a percentage change in odds, or a mean difference measured in natural units of outcome.

The synthesis will further be in form of summary of findings tables, simple graphs and forest plots as applicable using a STATA v16. This will follow the format of the Cochrane consumers and communication review group (32). We shall describe the included articles, group articles according to study design and type of intervention, organize and tabulate results to identify patterns and transform the results into a common descriptive format. These will be in form of outcome data tables, simple graphs and forest plots as applicable. These will feed into the summary of findings tables that inform the syntheses for sharing. We shall thus use both narrative and quantitative synthesis.

Sensitivity analysis

The sensitivity analysis will be done by removing studies from the meta- analysis one-by-one to see if the results of the meta-analysis are sensitive to any single study. We will also examine sensitivity of findings to risk of bias status (low risk, some concerns and high risk).

Ethics and dissemination

The review protocol was reviewed and approved by the School of Biomedical Science Research Ethics Committee, Makerere University College of Health Sciences (SBS-2022-213). The protocol was further cleared by Uganda National Council of Science and Technology, UNCST. Results will be disseminated through conference presentations and publication in peer-reviewed journal.

Patient and public involvement

Patients or the public will not be involved in the design, or conduct, or reporting, or dissemination plans of our research.

DISCUSSION

The accessibility to quality and efficacious antimalarial medicines is fundamental towards successful malaria treatment. However, this may be compromised by the inaccessibility and high cost of these antimalarials in communities in addition to the rising rate of resistance. There is therefore need to accelerate research in discovery and development of novel efficacious and less toxic antimalarials.

Herbal extracts have proven to contain various phytochemicals which have pharmacological properties. Globally, researchers have carried out primary studies that have documented and provided knowledge on the anti-plasmodial activities of numerous plants. This systematic review and evidence and gap map will therefore collate and map the available evidence, identify the gaps, and synthesize the efficacy of herbal antimalarial medicines in malaria affected regions globally in comparison with the recommended artemisinin on market. This will help collate evidence on the most efficacious herbal extract that can be used to inform the antimalarial drug development process.

Abbreviations

IC_{50}, Half-maximal inhibitory concentration

$RSA_{0\text{-}3h}$, Ring-stage survival assay

$TSA_{50,}$ Trophozoite Survival Assay

Author contributions

Conceptualization of the study was done by (MO, NL, KOO). MO, NL and KOO drafted the protocol, Critical review (MO, NL, KOO, AAK, RA, EAO), and approval of the final version (MO, NL, KOO, AAK, RA, EAO).

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Competing interests

None declared.

Patient consent for publication

Not applicable

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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Reported on Page # (Line Number)
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	**1(1)**
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	**NA**
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	**1(38); 3(121-122)**
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	**10 (417-430); 1(9-12)**
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	**10(432-435)**
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	**NA**
Support:			
Sources	5a	Indicate sources of financial or other support for the review	**10(438-441)**
Sponsor	5b	Provide name for the review funder and/or sponsor	**10(438-441)**
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	**10(438-441)**
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	**2(83-93)**
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	**4-5(165-173)**
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	**6(191-211)**
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	**6(213-225)**
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	**6-7(227-261)**

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	**7(279-286)**
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	**7(279-286)**
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	**7(289-295)**
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	**8(287-301)**
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	**5-6(175-189)**
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	**8(303-323)**
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	**9(371-385)**
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	**8(344-349)**
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	**9(388-391)**
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	**8(325-336)**
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	**8(338-342)**

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

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