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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

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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 Moses Ocan^{1,2}, Nakalembe Loyce^{1,3}, Kevin Ouma Ojiambo^{1,4}, Alison A. Kinengyere^{1,4}, Robert Apunyo¹, Ekwaro A. Obuku^{1,5,6} Correspondence: Dr. Moses Ocan, Africa Center for Systematic Reviews and Knowledge Translation, Makerere University College of Health Sciences moses.ocan@mak.ac.ug, Department of Pharmacology, School of Biomedical sciences, College of Health Sciences, Makerere University, P.O Box 7072, Kampala, Uganda 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 IC₅₀, RSA_{0-3h}, TSA₅₀ 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

54 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 Chinese herbal medicine, Artemisia annua from а 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).

8182 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.

94 Several reviews have been conducted documenting information on different plants for their 95 anti-plasmodial and antimalarial activities. (11-13). However, these reviews focused on 96 studies that investigated *In-vitro* and *In-vivo* activities of these herbs without comparing 97 their efficacies with the already existing modern anti-malarial medicines. Our current 98 review seeks to collate and map evidence comparing the antimalarial efficacy of herbal 99 medicines with that of artemisinin agents in malaria affected regions globally.

101 How the intervention might work

102 Early detection and treatment remain the principal strategies for control of malaria globally.

103 Treatment of malaria currently involves the use artemisinin combination therapies as

104 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 (<u>https://www.crd.york.ac.uk/prospero/</u>) 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 (IC₅₀, RSA_{0-3hrs}, TSA₅₀, parasite clearance rate) (Table 1). st review only

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

Intervention Domains	Indicators	Description
• Medicinal plants	 Kind/type of plant (shrub, herb, tree) Parts of the plant used (leaves, back, roots, shoots) 	 The type of plant, including geographical location 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 Clinical outcomes 	IndicatorsFever	DescriptionRelief 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 _{0-3hrs}	Concentration killing early- stage parasites (0-3hrs)
	• TSA_{50}	• Concentration killing 50% of trophozoites

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160 **Review question**

161 What is the *In-vitro* and *In-vivo* efficacy of anti-malarial herbal medicines used by
162 communities in malaria affected regions globally?
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Table 2: PICOST model for the review question

Element of PICO	Description		
Population (P)	Clinical: Malaria patients (children and adults)		
	Laboratory: Malaria parasites, <i>Plasmodium spp (e.g: P.</i>		
	<i>falciparum</i>). Wild or field strain parasites		
Intervention/Exposure	Clinical: Use of herbal medicines for management of		
r i i i i i i i i i i i i i i i i i i i	symptoms of malaria among children and adult patients		
	Lab: Plant extracts used in <i>in-vitro</i> assays against		
	Plasmodium parasites		
Comparator	• In-vitro (laboratory) studies: Artemisinin antimalarial		
Computator	agents		
	In vivo (clinical) studios: Nono		
Outcomes	Clinical: symptom resolution (fever, nausea/vomiting, joint		
	pains, fatigue/malaise, loss of appetite)		
	Laboratory: IC ₅₀ , RSA _{0-3h} , 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 (hina)		

Outcomes

- Primary outcomes
 - Clinical: symptom resolution (fever, nausea/vomiting, joint pains, fatigue/malaise, loss of appetite)
 - Laboratory: IC₅₀, RSA_{0-3h}, TSA₅₀, 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

178 Eligibility criteria

179 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

190 Exclusion criteria

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

³ 197 Identification of articles

5 198 **Data Sources**

- 7 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
- 18 208 articles will be screened for potential articles for inclusion in the review.

210 Search Strategy

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

Population:

- 214 <u>Laboratory</u>: Plasmodium falciparum, malaria parasites, Plasmodium species, Plasmodium spp,
 215 wild type parasites, field parasites, field isolates
- 28 215 What type parasites, field parasites,
 29 216 <u>Clinical:</u> Children, infants, adults
- ³⁰ 217 <u>Disease/condition:</u> Malaria, fever, malaria fever, malaria symptomatic patients, Plasmodium
 - 218 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:

- 226 <u>*Clinical</u>*: None</u>
- *Laboratory:* Dihydroartemisinin, Lumefantrine, Piperaquine

230 Outcome:

- 231 <u>*Clinical:*</u> Fever, nausea, vomiting, joint pains, malaise, loss of appetite, symptom resolution,
 232 symptom clearance
- Laboratory: IC₅₀, RSA_{0-3hrs}, TSA₅₀, 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. 7 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).

259 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.

266 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, IC₅₀, RSA_{0-3hrs}, TSA₅₀, 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.

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Heterogeneity
The I²-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 betweenstudy variation (21). Heterogeneity will be categorized as, low (I²=25%) (low), moderate (I²
=50) and high (I² >75%). Sub-group analysis will be done among articles categorized as low
and moderate heterogeneity.

7 **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).

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Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.	Erasmushogeschool	Open: first published as 10.1136/bmjopen-2022-069771 on 7 July 2023. Downloaded from http://bmjopen.bmj.com/ on June 11, 2025 at Department GEZ-LTA

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 IC₅₀, Half-maximal inhibitory concentration RSA_{0-3h}, Ring-stage survival assay TSA₅₀, Trophozoite Survival Assay **Author affiliations** 1. Africa Centre for Systematic Reviews and Knowledge Translation, College of Health Sciences, Makerere University, Kampala, Uganda 2. Department of Pharmacology, School of Biomedical Sciences, College of Health Sciences, Makerere University, Kampala, Uganda 3. Department of Pharmacology, College of Health Sciences, Soroti University, Soroti, Uganda 4. Albert Cook Library, College of Health Sciences, Makerere University, Kampala, Uganda 5. Clinical Epidemiology Unit, Department of Medicine, College of Health Sciences, Makerere University, Kampala, Uganda 6. Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK. **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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13	410	
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16	412	The authors have no conflict of interest to declare.
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19 20	415	None declared.
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22	417	Patient consent for publication
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24 25	419	
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PRISMA-P (Prefe address in a syste	erred matio	BMJ Open BMJ Open Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recomm c review protocol*	ended items to
Section and topic	Item No	Checklist item	Reported on Page (Line Number)
ADMINISTRATIVE	INFC		
Fitle: Identification	1a	Identify the report as a protocol of a systematic review	1(1)
	10	If the protocol is for an update of a previous systematic review, identify as such	NA 1(22.24)
Agistration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	1(23-24)
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mathing 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, identity 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, and reventions, comparators, and outcomes (PICO)	4-5(160-166)
METHODS		gies 202	
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, the light 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	6-7(210-257)

3 of 13			BMJ Open BMJ Open op op	
			rright, in	
	Study records: Data	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review b	7(250-257)
	Selection	11b	State the process that will be used for selecting studies (such as two independent reviewers) through $\frac{1}{2}$ ch 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) 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 a training and 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 the 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 $\overline{\mathbf{d}}$ had ding data and methods of combining data from studies, including any planned exploration of consistency (such as I $\overline{\mathbf{d}}$ Kendall's τ)	7(295-300)
		15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regresed on be	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 recommend the items. Amendmend distributed under a Cr	nende its to a reative	d that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite shere available) for import review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P (Commons Attribution Licence 4.0.	ant clarification on Group and is
	From: Shamseer L, M meta-analysis protoco	loher I ols (PF	D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systems f	ematic review and
			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	

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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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Primary Subject Heading :	Infectious diseases
Secondary Subject Heading:	Infectious diseases, Pharmacology and therapeutics
Keywords:	PARASITOLOGY, Herbal medicine < THERAPEUTICS, Pharmacology < TROPICAL MEDICINE, Tropical medicine < INFECTIOUS DISEASES, Diagnostic microbiology < INFECTIOUS DISEASES, Malaria



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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30 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) antimalarial 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 IC₅₀, RSA_{0-3h}, TSA₅₀, and Adequate Clinical and Parasitological response.

51 Ethics and dissemination: The review protocol was approved by the School of Biomedical

52 Science Research Ethics Committee, Makerere University College of Health Sciences (SBS-53 2022-213).

- 54 PROSPERO Registration Number: CRD42022367073
- 55 Key words: Herbal medicines, Antimalarial, Plasmodium falciparum, malaria, herbal extracts

57 Article summary58

59 Strengths and limitations of this study

60 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.

67 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.

72 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. SubSaharan 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).

99 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 currentefforts 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.

14 ¹¹³ 15 116

5 117 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.

132 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.

- 137 This protocol was registered in PROPSERO (<u>https://www.crd.york.ac.uk/prospero/</u>) registration
 138 number CRD42022367073 (16)

140 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 (IC₅₀, RSA_{0-3hrs}, TSA₅₀, parasite clearance rate) (Table 1).

Table 1: Intervention	autoomo domains for the ovidence a	nd gan man
 Medicinal plants 	Kind/type of plant (shrub, herb,	• The type of plant, including
	 Parts of the plant used (leaves, back, roots, shoots) 	 geographical location Plant material used, eithe entire plant or its parts (single
	• Name of the plant species	or combined)Include both Genus and
	• Age of the plant/part	 Either young or mature/ol-
• 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 o the plant, proportion of plan and solvent used
	• Ether extract	• Extraction time, extraction temperature, granulometry o the plant, proportion of plan and solvent used
	Chloroform extract	• Extraction time, extraction temperature, granulometry o the plant, proportion of plan and solvent used
	• Ethanolic extract	• Extraction time, extraction temperature, granulometry o the plant, proportion of plan and solvent used
	• Crude extract	• Proportions of the different extracts in the mixture
Outcomes Domains Clinical 	Indicators Fever 	DescriptionRelief from fever
ouicomes	 Body aches/malaise Joint pains Nausea/vomiting 	Relief from body achesRelief from joint painsRelief from vomiting

	parasitological response	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

182 What is the clinical, *In-vitro* and *In-vivo* efficacy of anti-malarial herbal medicines used by 183 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, <i>Plasmodium spp (e.g: P.</i>
	<i>falciparum</i>). Wild or field strain parasites
	In-vivo (animal) studies: Malaria parasites Plasmodium spo
	(e.g.P. herghei)
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
	• <i>In-vivo (animal) studies:</i> Artemisinin antimalarial agents
	Clinical studies: Antomicinis lass denting lanislasses
	• Chinical studies. Alternisinin based antimalarial agents
	• For <i>in-vitro</i> 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 sph clearance Plasmodium falcingrum clearance
Setting	Malaria affected countries globally (tropical countries)
~~~~·······S	(uopieur countries)

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	Designs: Cross-sectional studies, Randomized controlled trials (RCTs)
Time	2000 to date (Introduction of Artemisinin in malaria treatment outside China)
Outcomes	
Primary outcomes	
Clinical: <i>Plasmo</i> response, ACPR) loss of appetite)	<i>dium falciparum</i> parasite clearance (Adequate clinical and parasitological , symptom resolution (fever, nausea/vomiting, joint pains, fatigue/malaise,
• <i>In-vitro</i> studies: I	$C_{50}$ , $RSA_{0-3h}$ , $TSA_{50}$
• <i>In-vivo</i> (animal)	studies: _Parasite suppression rate, Plasmodium spp clearance
Secondary outcome	
• Rate of symptom	resolution (in-vivo and clinical studies)
• Safety of antiin teratogenicity, can drug reactions)	nalarial herbal medicines; <i>in-vivo</i> studies (LD50, organ toxicity, rcinogenicity, mutagenicity); clinical studies (reported side effects/adverse
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	t of malaria symptoms
• Peer reviewed	articles reporting on <i>in-vitro</i> efficacy of anti-malarial herbal medicines
• Peer reviewed	articles reporting on <i>in-vivo</i> efficacy of anti-malarial herbal medicines
Peer reviewed	articles published from the years 2000 to date
• Articles publis	hed in both English and non-English language
Evaluation outtouto	
Exclusion criteria	
• Peer reviewed	articles whose full text cannot be retrieved. Efforts will be made to retrieve $II$ text exticles before prior to being evolved. The librarian (AAK) will use
all included ful	in text articles before prior to being excluded. The librarian (AAK) will use
addition AAK	will contact other librarians for retrieval of full text articles. However, full
text articles w	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
	articles from studies that did not receive ethical review and approval
Identification of artic	les
Data Sources	
Article search will be	e performed by an experienced librarian (AAK) in PubMed MEDLINE
Ovid. EMBASE and V	Veb of Science, and other grev literature sources Medical subject headings
(MeSH) terms and Bo	olean operators "AND" and "OR" will be used during article search. The
data sources will inch	ide scholarly databases, grev literature sources, contacting authors as well
as screening reference	ce sources of included studies. we shall search grev literature from
organization websites	such as WHO, Medicines for malaria venture, institutional repositories.
and contact experts/r	esearchers 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

243 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:**

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

254 <u>Clinical:</u> Children, infants, adults

may not be identified by the search.

255 <u>Disease/condition:</u> Malaria, fever, malaria fever, malaria symptomatic patients, Plasmodium
 256 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:** 
  - <u>Clinical</u>: Artemisinin based antimalarial agents

Laboratory: Dihydroartemisinin, Lumefantrine, Piperaquine

#### 268 Outcome:

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

*Laboratory:* IC₅₀, RSA_{0-3hrs}, TSA₅₀, 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 xaxis. 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.

# 294295 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).

#### 5 303 5 304

## 305 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.

#### 313 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,
IC₅₀, RSA_{0-3hrs}, TSA₅₀, malaria parasite suppression rate) as illustrated in the PICOST (Table 2)

#### 319 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)

#### 354 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.

#### 360 Heterogeneity

The I²-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 (I²=25%) (low), moderate (I² =50) and high (I² >75%). Sub-group analysis will be done among articles categorized as low and moderate heterogeneity.

#### 367 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.

#### 377 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)

#### 387 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.

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404 Sensitivity analysis

 405 The sensitivity analysis will be done by removing studies from the meta- analysis one-by-one to
406 see if the results of the meta-analysis are sensitive to any single study. We will also examine
407 sensitivity of findings to risk of bias status (low risk, some concerns and high risk).
408

410 Ethics and dissemination

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

#### **Patient and public involvement**

418 Patients or the public will not be involved in the design, or conduct, or reporting, or419 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. 

- 48 435 Abbreviations
- 49
  50 436 IC₅₀, Half-maximal inhibitory concentration
  51
- ⁵² 437 RSA_{0-3h}, Ring-stage survival assay
  - 438 TSA₅₀, Trophozoite Survival Assay

# 439 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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The review is supported by funds from the Ministry of Science, Technology and Innovations grant number STI/SIVC/49/2022. The funders do not have any role in the design, conduct, and interpretation of the findings of the study.

#### **Conflict of interest**

452 The authors have no conflict of interest to declare.

2		
3	453	
4 5	454	Competing interests
6	455	None declared.
7	456	
8	457	Patient consent for publication
9	458	Not applicable
10	450	Not applicable
12	459	
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Section and topic	Item No	Checklist item	Reported o (Line Nu
ADMINISTRATIV	E INF	ORMATION	~
Title:		Era Iate	
Identification	1a	Identify the report as a protocol of a systematic review	1(
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	1(38); 3(1
Authors:		and the sector of the sector o	
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mating address of corresponding author	10 (417-43)
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	10(432
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	N
Support:			
Sources	5a	Indicate sources of financial or other support for the review	10(438
Sponsor	5b	Provide name for the review funder and/or sponsor	10(438
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
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	2(83-
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participents, anterventions, comparators, and outcomes (PICO)	4-5(165
METHODS		gies	
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-
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, that registers or other grey literature sources) with planned dates of coverage	6(213
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limit such that it could be repeated	6-7(22)

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Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review ding to the second se	7(279-286)
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through sch ph review (that is, screening, eligibility and inclusion in meta-analysis)	ase of the <b>7(279-286)</b>
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently in dup processes for obtaining and confirming data from investigators	plicate), any 7(289-295)
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources) assumptions and simplifications	anned data 8(287-301)
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and at the sought outcomes for which data will be sought, including prioritization of main and at the sought of the sou	atcomes, <b>5-6(175-189)</b>
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether the solution outcome or study level, or both; state how this information will be used in data synthesis	lone at the 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 a handling methods of combining data from studies, including any planned exploration of consistency (such as I Kend	data and <b>8(344-349)</b> dall's τ)
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regreszion	9(388-391)
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned $\frac{1}{2}$	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting studies)	within <b>8(325-336)</b>
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 recom the items. Amendmen distributed under a Ca	mende nts to a reative	ed that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite scherogavaila a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) if held by the commons Attribution Licence 4.0.	ble) for important clarification on PRISMA-P Group and is
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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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Date Submitted by the Author:	10-Mar-2023
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<b>Primary Subject Heading</b> :	Infectious diseases
Secondary Subject Heading:	Infectious diseases, Pharmacology and therapeutics
Keywords:	PARASITOLOGY, Herbal medicine < THERAPEUTICS, Pharmacology < TROPICAL MEDICINE, Tropical medicine < INFECTIOUS DISEASES, Diagnostic microbiology < INFECTIOUS DISEASES, Malaria



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 Moses Ocan ^{1,2} , Nakalembe Loyce ^{1,3} , Kevin Ouma Ojiambo ^{1,5} , Alison A. Kinengyere ^{1,4} , Robert Apunyo ¹ , Ekwaro A. Obuku ^{1,5,6} Author affiliations
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Makerere University, P.O Box 7072, Kampala, Uganda
<b>ABSTRACT</b> <b>Introduction</b> : 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.
<b>Methods and analysis:</b> 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 ( <i>In-vitro</i> studies), Newcastle Ottawa tool (Observational studies) and SYRCLE's risk of bias tool for animal studies ( <i>In-vivo</i> 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 _{0-3h} , TSA ₅₀ .
<b>Ethics and dissemination:</b> 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

- Article summary

56 Strengths and limitations of this study

#### 57 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.

#### 64 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.

#### 69 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). 

## 96 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 (IC₅₀, RSA_{0-3hrs}, TSA₅₀, parasite clearance rate) (Table 1). 

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21 22	174	Table 1: Intervention-	outcome domains for the evidence a	nd gap map
23 24		Intervention Domains	Indicators	Description
25 26		• Medicinal plants	• Kind/type of plant (shrub, herb, tree)	• The type of plant, including geographical location
27 28			• Parts of the plant used (leaves,	• Plant material used, either
29 30			back, roots, shoots)	entire plant or its parts (single or combined)
31 32 33			• Name of the plant species	• Include both Genus and species names
34 35			• Age of the plant/part	• Either young or mature/old plant/or parts
36 37 38 39		• Plant extract	• Water extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
40 41 42 43 44			Methanol extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
45 46 47 48 49			• Ether extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
50 51 52 53			Chloroform extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
54 55 56 57 58			• Ethanolic extract	• Extraction time, extraction temperature, granulometry of the plant, proportion of plant and solvent used
59 60			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
			<ul> <li>Joint pains</li> <li>Nausea/vomiting</li> </ul>	Relief from vomiting
			Appetite	<ul> <li>Gaining of appetite</li> </ul>
			<ul> <li>Adequate clinical and parasitological response (17)</li> </ul>	<ul> <li>Resolution of clinical symptoms of malaria and Plasmodium parasite</li> </ul>

clearance

• Laboratory	• IC ₅₀ , • Inhibitory concentration killing 50% of parasites
oncomes	<ul> <li>RSA_{0-3hrs}</li> <li>Concentration killing early stage parasites (0-3hrs)</li> </ul>
	TSA ₅₀ Concentration killing 50% of trophozoites
<b>Review question</b> What is the clinical, communities in malaria	<i>In-vitro</i> and <i>In-vivo</i> efficacy of anti-malarial herbal medicines used by a affected regions globally?
Table 2: PICOST mo	del for the review question
Element of PICO	Clinical: Malaria natients (children and adults)
	In-vitro studies: Malaria parasites, <i>Plasmodium spp (e.g: P. falciparum)</i> . Wild or field strain parasites In-vivo (animal) studies: Malaria parasites, <i>Plasmodium spp</i>
Intervention/Exposur	(e.g.P. bergnet)         'e       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
	<i>In-vivo studies:</i> Plant extracts used against <i>Plasmodium</i> parasite infections of laboratory animals
Comparator	• In-vitro studies: None
	<ul> <li>In-vivo (animai) stuates: Artemisinin antimatarial agents</li> <li>Clinical studies: Artemisinin based antimalarial agents</li> </ul>
	• For <i>in-vitro</i> 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,
Setting	Plasmodium spp clearance, Plasmodium falciparum clearanceMalaria affected countries globally (tropical countries)
Setting	Plasmodium spp clearance, Plasmodium falciparum clearanceMalaria affected countries globally (tropical countries)Designs: Cross-sectional studies, Randomized controlled trials (RCTs)

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109	Clinical: <i>Plasmodium falsingnum</i> peresite algerance (Adaguste alinical and peresitelegical)		
190	• Chinical. <i>Flasmoulum faicipurum</i> parasite clearance (Adequate chinical and parasitological response. ACPR), sumptom resolution (favor, nausoa/vomiting, joint nains, fatigua/malaisa		
191	less of appointe)		
192	loss of appende)		
193	a la vitue studiose IC DCA TCA		
194 105	• <i>In-vitro</i> studies: $IC_{50}$ , $KSA_{0-3h}$ , $ISA_{50}$		
195 106	• In vive (animal) studios: Deregite suppression rate. Plasmodium and classenes		
196	• <i>In-vivo</i> (animal) studies: _Parasite suppression rate, Plasmodium <i>spp</i> clearance		
197			
198	Secondary outcome		
199	• Safety of antimalarial herbal medicines; <i>in-vivo</i> studies (LD50, organ toxicity,		
200	teratogenicity, carcinogenicity, mutagenicity); clinical studies (reported side effects/adverse		
201	drug reactions)		
202			
203	Eligibility criteria		
204	Inclusion criteria		
205	• Peer reviewed articles reporting clinical efficacy of herbal medicines in malaria treatment		
206	among children and adults in malaria affected regions globally		
207	• Peer reviewed articles reporting on herbal medicinal plants used by communities for		
208	management of symptoms of malaria in malaria affected regions globally		
209	• Peer reviewed articles reporting on the safety of herbal medicines used by communities		
210	in management of malaria symptoms		
211	• Peer reviewed articles reporting on <i>in-vitro</i> efficacy of anti-malarial herbal medicines		
212	• Peer reviewed articles reporting on <i>in-vivo</i> efficacy of anti-malarial herbal medicines		
213	<ul> <li>Peer reviewed articles published from the years 2000 to date</li> </ul>		
214	• Articles published in both English and non-English language		
215			
216	Exclusion criteria		
217	• Peer reviewed articles whose full text cannot be retrieved. Efforts will be made to retrieve		
218	all included full text articles before prior to being excluded. The librarian (AAK) will use		
219	external sources like Web of Science, EMBASE, Sci-Hub, Lib-Hub and PDF Drive. In		
220	addition, AAK will contact other librarians for retrieval of full text articles. However, full		
221	text articles which will still not be able to be accessed despite these efforts will be		
222	excluded from the review.		
223	• Peer reviewed articles from studies that did not receive ethical review and approval		
224			
225	Identification of articles		
226	Data Sources		
227	Article search will be performed by an experienced librarian (AAK) in PubMed. MEDLINF		
228	Ovid, EMBASE and Web of Science, and other grey literature sources. Medical subject headings		
229	(MeSH) terms and Boolean operators "AND" and "OR" will be used during article search. The		
230	data sources will include scholarly databases, grey literature sources, contacting authors as well		
231	as screening reference sources of included studies. we shall search grev literature from		
232	organization websites such as WHO, Medicines for malaria venture. institutional repositories		
233	and contact experts/researchers in malaria field. We will also search Google Scholar for		
234	additional studies that may be missed from the other sources. The search will be limited to 2000		
235	to 2022.		
236	We will also screen through reference lists of included studies for additional eligible studies that		
237	may not be identified by the search.		

#### **Search Strategy** 240 Full article search

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:**

247 <u>Laboratory:</u> Plasmodium falciparum, Plasmodium species, wild type plasmodium parasites, field
248 plasmodium parasites, laboratory animals (Wister albino rats, Mice).

# 250 <u>Clinical:</u> Children, infants, adults

251 <u>Disease/condition:</u> Malaria, fever, malaria fever, malaria symptomatic patients, Plasmodium
 252 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:**

260 <u>*Clinical: Artemisinin based antimalarial agents*</u>

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

#### **Outcome:**

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

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

272 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 xaxis. 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.

#### 292 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**

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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, IC₅₀, RSA_{0-3hrs}, TSA₅₀, 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)

# 353 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.

#### 359 Heterogeneity

The I²-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 (I²=25%) (low), moderate (I² =50) and high (I² >75%)_(29). Sub-group analysis will be done among articles categorized as low and moderate heterogeneity.

#### 368 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.

#### 378 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:

$c_{\alpha}(d) =$	4	_ d ²
$se(a) = \sqrt{2}$	N	+ 2N

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

#### 388 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.

7

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
- IC₅₀, Half-maximal inhibitory concentration
- RSA_{0-3h}, Ring-stage survival assay
  - TSA₅₀, 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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$\begin{array}{c}1\\2\\3\\4\\5\\6\\7\\8\\9\\10\\11\\21\\3\\14\\15\\16\\17\\8\\9\\20\\21\\22\\32\\4\\25\\26\\7\\28\\29\\30\\31\\23\\34\\35\\36\\37\\38\\9\\40\\1\\42\\44\\45\\46\\47\\1\end{array}$	516 517 518 520 521 522 523 524 525 526 527 528 530 531 532 533 534 535 536 537 538 539	<ol> <li>Egger M, Davey Smith G Fau - Schneider M, Schneider M Fau - Minder C, Minder C. Bias in meta-analysis detected by a simple, graphical test. 1997(0959-8138 (Print)).</li> <li>Duval S, Tweedie R. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. 2000(0006-341X (Print)).</li> <li>Doleman BA-O, Freeman SA-O, Lund JN, Williams JP, Sutton AJ. Funnel plots may show asymmetry in the absence of publication bias with continuous outcomes dependent on baseline risk: presentation of a new publication bias test. (1759-2887 (Electronic)).</li> <li>Schünemann HJ, Cuello C, Akl EA, Mustafa RA, Meerpohl JJ, Thayer K, et al. GRADE guidelines: 18. How ROBINS-1 and other tools to assess risk of bias in nonrandomized studies should be used to rate the certainty of a body of evidence. 2013(1878-5921 (Electronic)).</li> <li>Higgins JP. Commentary: Heterogeneity in meta-analysis should be expected and appropriately quantified. 2009(1464-3685 (Electronic)).</li> <li>Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al. Cochrane handbook for systematic reviews of interventions: John Wiley &amp; Sons; 2019.</li> <li>López-López JA-O, Page MJ, Lipsey MW, Higgins JPT. Dealing with effect size multiplicity in systematic reviews and meta-analyses. LID - 10.1002/jrsm.1310 [doi]. (1759-2887 (Electronic)).</li> <li>Waddington HJ. Broadening horizons in impact evaluation for water, sanitation and hygiene planning: recycling and reinterpreting evidence: London School of Hygiene &amp; Tropical Medicine; 2021.</li> <li>Ryan R. Cochrane Consumers and Communication Review Group. 'Cochrane Consumers and Communication Review Group: data synthesis and analysis'. 2013.</li> </ol>
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address in a system	emat	d Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended review protocol*	nended item
Section and topic	Item No	Checklist item	Reported o (Line Nu
ADMINISTRATIV	E INF	ORMATION	× •
Title:		Era late	
Identification	1a	Identify the report as a protocol of a systematic review	1(
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	1(38); 3(
Authors:		ind is critical and is critica	
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mail address of corresponding author	10 (417-43)
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	10(432
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	N
Support:			
Sources	5a	Indicate sources of financial or other support for the review	10(438
Sponsor	5b	Provide name for the review funder and/or sponsor	10(438
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
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	2(83-
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, anterventions, comparators, and outcomes (PICO)	4-5(165
METHODS		gies 2022	
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-
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, that registers or other grey literature sources) with planned dates of coverage	6(213
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(22)

		BMJ Open BMJ Open-20	
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 get phare review (that is, screening, eligibility and inclusion in meta-analysis)	ase of the <b>7(279-286)</b>
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently in dup processes for obtaining and confirming data from investigators	licate), any <b>7(289-295)</b>
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources) assumptions and simplifications	anned data 8(287-301)
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and a to with rationale	itcomes, <b>5-6(175-189)</b>
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether the state how this information will be used in data synthesis	lone at the 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 a handling of methods of combining data from studies, including any planned exploration of consistency (such as I Kenda	data and 8(344-349) all's τ)
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regresion)	9(388-391)
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned $\frac{1}{2}$	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting studies)	within 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 recom the items. Amendmen distributed under a Ca	mende nts to a reative	ed that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when availat a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) if held by the commons Attribution Licence 4.0.	ble) for important clarification on the PRISMA-P Group and is
From: Shamseer L, N meta-analysis protoco	Ioher I ols (PI	D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred regorting RISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.	; items for systematic review and
		For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	