© 2026 Journal of Pharmacy & Pharmacognosy Research, 14 (2), 2495, 2026
ISSN 0719-4250
https://jppres.com

DOI: https://doi.org/10.56499/jppres_14.2.2495

Review

Antimalarial activity and toxicity of *Garcinia mangostana* L. pericarp extracts and major xanthones: A systematic review of *in vitro* and *in vivo* studies

[Actividad antipalúdica y toxicidad de los extractos del pericarpio de *Garcinia mangostana* L. y sus principales xantonas: una revisión sistemática de estudios *in vitro* e *in vivo*]

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Abstract

Context: Malaria morbidity remains high, and resistance to antimalarial drugs and diagnostics continues to expand.

Aims: To systematically evaluate the *in vitro*/*in vivo* antimalarial efficacy and toxicity of *Garcinia mangostana* L. pericarp extracts (MP)/fractions and major xanthones.

Methods: A PRISMA 2020 systematic review was performed and registered in PROSPERO. Searches were conducted in six databases up to March 30, 2025, using predefined keywords. Original English-language studies reporting the antimalarial activity and/or toxicity of MP extracts, fractions, or major xanthones *in vitro*/*in vivo* were included. Risk of bias was assessed using SciRAP (*in vitro*) and ToxRTool (*in vivo*). Due to methodological heterogeneity, findings were synthesized descriptively.

Results: From 3,785 records, 20 studies (16 *in vitro*; 4 *in vivo*) were included. EEs and non-polar fractions (e.g., dichloromethane, hexane) were frequently reported as active to very active *in vitro*. α-Mangostin (α-MG) exhibited activity against *Plasmodium falciparum* strains across assays (IC_{50} range: 2.2–36.1 µM), and intraperitoneal administration in rodent malaria models was associated with high chemosuppression (>80%) in a single study; oral activity was lower. Low acute toxicity was observed at high doses; however, chronic exposure was associated with hepatic and renal alterations in some studies. Limitations of the evidence: Reporting quality was variable. Although all *in vivo* studies were classified as reliable without restrictions, the risk of bias was frequently high *in vitro,* and reporting on cytotoxicity and selectivity was inconsistent across studies, limiting the ability to draw conclusions for clinical application.

Conclusions: MP-derived materials, particularly α-MG, exhibit experimental antimalarial activity. However, heterogeneity, incomplete reporting, and limitations regarding oral exposure warrant standardized studies on efficacy, selectivity, and pharmacokinetics.

Keywords: α-mangostin; antiplasmodial; antiprotozoal; fruit peel; toxicity.

Resumen

Contexto: La morbilidad por malaria sigue siendo alta y la resistencia a los fármacos antipalúdicos y a los métodos de diagnóstico continúa en aumento.

Objetivos: Evaluar sistemáticamente la eficacia y la toxicidad antipalúdicas, *in vitro* e *in vivo*, de los extractos y fracciones del pericarpio de *Garcinia mangostana* L. (MP) y de sus principales xantonas.

Métodos: Se realizó una revisión sistemática según las directrices PRISMA 2020, registrada en PROSPERO. Se realizaron búsquedas en seis bases de datos hasta el 30 de marzo de 2025, utilizando palabras clave predefinidas. Se incluyeron estudios originales en inglés que informaran sobre la actividad antipalúdica y/o la toxicidad de los extractos, fracciones o principales xantonas de MP, tanto *in vitro* como *in vivo*. El riesgo de sesgo se evaluó mediante SciRAP (*in vitro*) y ToxRTool (*in vivo*). Debido a la heterogeneidad metodológica, los hallazgos se sintetizaron de manera descriptiva.

Resultados: De 3785 registros, se incluyeron 20 estudios (16 *in vitro*; 4 *in vivo*). Los extractos etanólicos y las fracciones no polares (p. ej., diclorometano, hexano) se han reportado con frecuencia como activos o muy activos *in vitro*. α-Mangontina (α-MG) mostró actividad contra cepas de *Plasmodium falciparum* en diversos ensayos (rango de CI_{50}: 2,2–36,1 µM), y la administración intraperitoneal en modelos de malaria en roedores se asoció con una alta quimiosupresión (>80%) en un estudio; la actividad por vía oral fue menor. Se observó baja toxicidad aguda a dosis altas; sin embargo, la exposición crónica se asoció con alteraciones hepáticas y renales en algunos estudios. Limitaciones de la evidencia: La calidad de los informes fue variable. Si bien todos los estudios *in vivo* se clasificaron como fiables sin restricciones, el riesgo de sesgo fue frecuentemente alto *in vitro*, y la información sobre citotoxicidad y selectividad fue inconsistente entre los estudios, lo que limita la capacidad de extraer conclusiones para su aplicación clínica.

Conclusiones: Los materiales derivados de MP, en particular el α-MG, muestran actividad antipalúdica en estudios experimentales. Sin embargo, la heterogeneidad, la información incompleta y las limitaciones de la exposición oral justifican la realización de estudios estandarizados sobre la eficacia, la selectividad y la farmacocinética.

Palabras Clave: α-mangostina; antiplasmodial; antiprotozoario; cáscara de fruta; toxicidad.

ARTICLE INFO
Received: March 2, 2025.
Accepted: April 7, 2026.
Available Online: April 28, 2026.

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Abbreviations: α-MG: α-Mangostin; γ-MG: γ-Mangostin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BME: Ball milling extraction; BUN: Blood urea nitrogen; DCM: Dichloromethane; EAE: Ethyl acetate extract; EE: Ethanolic extract; FIC: Fractional inhibitory concentration; HIA: Hypoxanthine incorporation assay; HRE: Heat reflux extraction; IC_{50}: 50% inhibitory concentration; IC_{90}: 90% inhibitory concentration; ip: Intraperitoneal route; MAE: Microwave-assisted extraction; ME: Methanolic extract; MF: Mangosteen fruit; MP: Mangosteen pericarp; MPEE: Mangosteen peel ethanolic extract; MPME: Mangosteen peel methanolic extract; NA: Not available; NR: Not reported; pf: Plasmodium falciparum; pLDH: Plasmodium sp. lactate dehydrogenase; pLDH: Parasite lactate dehydrogenase; po: Oral route; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analysis; PROSPERO: International Prospective Register of Systematic Reviews; ROS: Reactive oxygen species; WHO: World Health Organization.

INTRODUCTION

Malaria remains a significant global health issue, with 263 million cases and 597,000 deaths reported in 2023, mainly in sub-Saharan Africa (WHO, 2024). The spread of pfhrp2 gene deletions, partial artemisinin resistance, piperaquine resistance, and growing insecticide resistance complicate control efforts and highlight the need for new therapies (Kiemde et al., 2017; WHO, 2024; Xie et al., 2020). Natural products have been key sources of antimalarial drugs, such as quinine from *Cinchona* bark (1820) and artemisinin from *Artemisia annua* (1972) (Dagen, 2020; Runhong & Daqing, 2020). While artemisinin-based combination therapies remain first-line treatments, resistance linked to Kelch 13 mutations and prolonged drug exposure makes finding new compounds with different mechanisms urgent (Erhirhie et al., 2021; Xie et al., 2020). The rising incidence of malaria and the emergence of drug-resistant Plasmodium strains necessitate the development of novel antimalarial agents (Agbodeka et al., 2016). One such herb is mangosteen (*Garcinia mangostana* L., *Clusiaceae*), renowned for its unique flavor and primarily cultivated in Southeast Asia (Yao et al., 2023). Its pericarp, which constitutes about 83% of the fruit and is often discarded, contains numerous bioactive compounds (Yuvanatemiya et al., 2022), such as anticancer (Taokaew et al., 2014), antioxidant (Suttirak & Manurakchinakorn, 2014), antimicrobial (Semangoen et al., 2025; Sitti et al., 2018), antimalarial (Abdillah et al., 2015), anti-aging (Widowati et al., 2020), and anti-inflammatory activities (Astuti et al., 2019).

MP is rich in secondary metabolites, particularly xanthones (Ong et al., 2020). Over 60 xanthone derivatives have been identified, including α-, β-, and γ-MG, gartanin, and garcinone E (Ong et al., 2020; Rizaldy et al., 2021). Some of these compounds exhibit antimalarial activity in laboratory and animal studies, possibly by inhibiting heme detoxification or targeting chloroquine-resistant *Plasmodium falciparum* strains (Kuncoro et al., 2018; Ibraheem et al., 2020; Mahabusarakam et al., 1987; Mamede et al., 2020; Upegui et al., 2015).

Research on MP extracts and xanthones has yielded variable results regarding potency, selectivity, and effectiveness in living organisms, depending on extraction methods, parasite strains, and test systems (Abdillah et al., 2015; Bunyong et al., 2014). Reports on toxicity and cytotoxicity are also inconsistent. To date, no systematic review has comprehensively synthesized the available evidence on the antimalarial activity and toxicity of MP extracts, fractions, and major xanthones. Therefore, a systematic consolidation of antimalarial efficacy, toxicity, and methodological quality is warranted.

The following review question was formulated using the PECO framework: Population—Plasmodium-infected cells or animals; Exposure—mangosteen (*Garcinia mangostana*) pericarp extracts, fractions, or major xanthones; Comparator—untreated controls or standard antimalarial drugs; Outcome—efficacy (*in vitro* or *in vivo* antiplasmodial activity) and toxicity. This review summarizes antimalarial findings for MP extracts, fractions, and major xanthones and assesses study reliability using SciRAP and ToxRTool.

MATERIAL AND METHODS

Review methodology

The protocol for this systematic review has been registered with PROSPERO (Prospective International Registration for Systematic Reviews), registration number: CRD420250651010. Minor deviations from the initial protocol were made during the screening phase and are described in the Methods section. This systematic review was conducted in accordance with the PRISMA 2020 guidelines (Page et al., 2021).

Inclusion criteria

The inclusion criteria for this study were limited to research articles available in full text with accessible author information and published in English. Additionally, this study included not only reports on the results of *in vitro* and *in vivo* antimalarial tests of extracts, fractions, or major xanthone compounds derived from MP but also the outcomes of toxicity tests of mangosteen fruit peel extracts. Only articles accessible as of March 30, 2025, were considered for inclusion.

Information sources

The data used in the preparation of this systematic review were obtained from Web of Science,

ScienceDirect, PubMed, Google Scholar, Scopus, and Wiley Online Library.

Search strategy

Keywords used in the literature search included antimalarial, malaria, antiplasmodial, mangosteen, *Garcinia*, *G. mangostana*, mangostin, gartanin, and xanthone. Studies on the toxicity and safety of the extracts were searched for separately. Boolean operators, such as "AND" and "OR," were used to maximize search results. Additionally, the researchers used the MeSH Term feature in PubMed (https://www.ncbi.nlm.nih.gov/mesh/) to identify relevant keyword synonyms. The use of the asterisk (*) symbol further facilitates the retrieval of related articles based on the entered keywords. Filters were applied to the literature search to limit results to journal articles only, thereby excluding non-journal publications such as books, conference proceedings, and other irrelevant materials. The specific keywords used in this search strategy are shown in Annex 3.

Additional literature was also searched to support the discussion regarding the mechanism of action, pharmacokinetics, and toxicological aspects of xanthone compounds. This literature was obtained through additional searches in the same databases and by reviewing the reference lists of relevant articles. These additional articles were not included in the systematic review's screening, eligibility assessment, or data extraction processes. However, they were used as supporting literature to provide a scientific context in the discussion of the research findings.

Selection process

The screening process—which included the removal of duplicates, evaluation of titles/abstracts, and assessment of full-text articles based on inclusion criteria—was conducted independently by two reviewers using Rayyan (https://new.rayyan.ai/), a systematic review software platform, to ensure methodological rigor and minimize bias. Disagreements were resolved through discussion and, when necessary, consultation with a third reviewer. Inter-reviewer agreement was evaluated using Cohen's kappa coefficient, which indicated substantial agreement between reviewers (κ = 0.63).

Data collection process

The study title, article citation, country of origin, study design, assay name, tested compounds/extracts/fractions, plant source, subject or target assay, reported antimalarial activity (e.g., IC_{50} in µg/mL or µM, or percentage inhibition), comparators or positive controls, control activity values (IC_{50}), cytotoxicity assay methods, cytotoxicity results (IC_{50}), and SI were extracted. If relevant data were missing or unclear, studies were included only when sufficient information could be obtained from the text, tables, or figures; otherwise, exclusion from quantitative analysis but retention for qualitative synthesis was applied. No statistical imputation was performed.

Data items (outcomes)

The primary outcome evaluated in this analysis was antimalarial activity. In *in vitro* studies, activity was measured as IC_{50}, with values expressed in µg/mL for plant extracts and fractions (Ouattara et al., 2006) and in µM for isolated single compounds (Batista et al., 2009). In *in vivo* studies, outcomes were evaluated by the percentage reduction in parasitemia (Upegui et al., 2015).

Assessment of risk of bias

The methodological quality of *in vivo* studies was carefully evaluated using the ToxRTool (Schneider et al., 2009). This assessment considered the clarity with which test interventions were identified, the characterization of test organisms, the transparency of the study design, the rigor in reporting results, and the consistency between the study design and outcomes. In parallel, *in vitro* studies were appraised using the SciRAP web platform (www.scirap.org), with particular attention to methodological robustness, completeness of reporting, and relevance to the study objectives (Roth et al., 2021). Studies deemed to have low reliability typically lacked adequate descriptions of test substances, provided incomplete information regarding experimental or assay conditions, omitted appropriate controls, offered limited details about randomization or blinding procedures, failed to specify concentration ranges, or inadequately reported the number of replicates and statistical analyses.

Assessment of the certainty of evidence
A simplified certainty framework was applied based on three domains: risk of bias (RoB), consistency of results, and directness of evidence. RoB was assessed using SciRAP (*in vitro*) and ToxRTool (*in vivo*). Consistency was evaluated based on the similarity of effect estimates across comparable studies, and directness was assessed based on the relevance of the models (*in vivo* > *in vitro*). Overall certainty was classified as high, moderate, low, or very low. Downgrading of certainty was primarily driven by indirectness (animal models), a limited number of studies, and inconsistency, rather than risk of bias in *in vivo* studies.

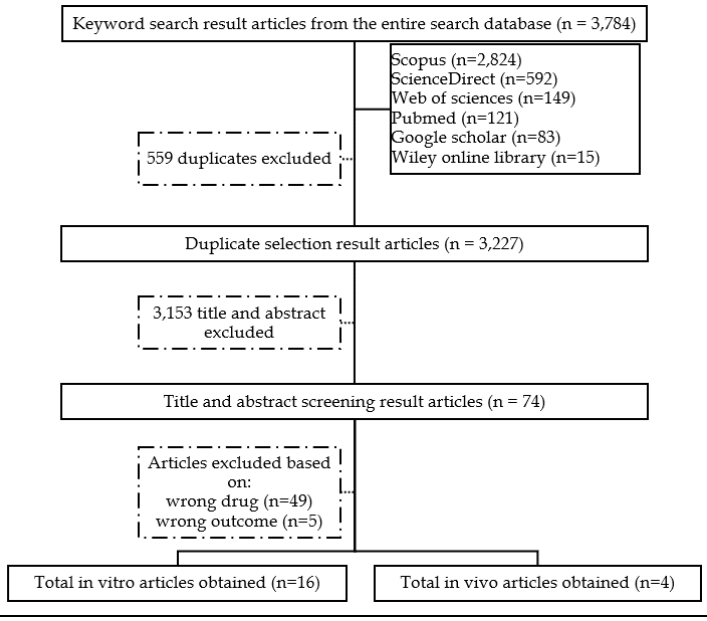


Figure 1. PRISMA flow diagram of study selection.

Synthesis methods

Eligibility for synthesis

Studies were considered eligible if they evaluated extracts, fractions, or major xanthone compounds derived from mangosteen peel, with exceptions applied to studies with methodological ambiguities.

Data preparation for synthesis

Activity data were standardized (e.g., conversion of units to µg/mL or µM) and transcribed without imputation. Potency was classified according to Ouattara et al. (2006) as highly active (IC_{50} < 5 µg/mL), active (5–50 µg/mL), or weak (50–100 µg/mL) for extract or fraction. For individual compounds, classification follows the criteria proposed by Batista et al. (2009), where activity was categorized as potent (IC_{50} < 1 µM), promising (1 < IC_{50} ≤ 20 µM), moderate (20 < IC_{50} ≤ 100 µM), low (100 < IC_{50} ≤ 200 µM), and inactive (IC_{50} > 200 µM). In *in vivo* studies, the therapeutic response was categorized as high if the chemosuppression value was >80%, moderate if it was 50–80%, and low if it was <50% (Upegui et al., 2015).

Grouping and graphical methods

Data from the included articles were organized into several tables and one heatmap. Missing data were handled using standardized labels. NA was used when a parameter was not applicable or could not exist based on the study design (e.g., Tmax for intravenous administration). NR was used when a parameter was expected to be measured but was not reported in the original study (e.g., CC_{50} was not reported despite a cytotoxicity assessment).

The study characteristics table lists the studies used in this review. The *in vitro* study risk of bias table was compiled based on the results of the critique of each article, calculated according to Annex 4. The extract/fraction study table was created to examine the antimalarial activity of extracts across assays, solvent types, and extraction methods. The α-MG pharmacokinetic table was created to examine the *in vivo* pharmacokinetic parameters of α-MG across formulations/vehicles, species, and routes of administration.

Qualitative α-MG activity data were converted into ordinal scores (0–3), with the categories potent (3), promising (2), moderate (1), and not available (0). Visualization was performed using a heatmap to evaluate α-MG activity patterns against various *P. falciparum* strains across different test methods.

Statistical synthesis methods

A meta-analysis was deemed inappropriate due to methodological heterogeneity, including variations in *Plasmodium* strains (e.g., *P. berghei* vs. Pf) and extraction protocols. Instead, a descriptive synthesis was performed to examine the antimalarial activity of MP extracts and major xanthone compounds in the extracts across various *Plasmodium* strains and assays.

RESULTS

Study selection

Study flowchart

The selection process in this article followed the PRISMA flowchart (Fig. 1).

Excluded studies

Five exclusion criteria were applied: 1) inappropriate study design (n = 2,850); 2) irrelevant compounds (n = 260); 3) non-targeted outcomes (n = 92); 4) ineligible publication formats (n = 3); and 5) publications in languages other than English (n = 2).

Study characteristics

Variability in antimalarial activity across studies is influenced by plant origin, solvent, and extraction method, as well as parasite strain and assay type, as shown in Table 1.

Risk of bias in the studies

Based on methodological quality assessment, 2 out of 19 studies (10.5%) were classified as high risk of bias (<50%), 5 studies (26.3%) as moderate risk (50–74%), and the majority, 12 studies (63.2%), as low risk (≥75%) (Table 2). Based on the ToxRTool evaluation, four *in vivo* studies reviewed in this article were categorized as reliable without restrictions (Annex 5).

Synthesis results

Characteristics of the contributing studies

α- and γ-MG compounds, gartanin, and other xanthones isolated from plant sources other than *G. mangostana* were also analyzed based on the assumption that these compounds can mimic the antimalarial efficacy of xanthones isolated from MP.

DISCUSSION

Antimalarial testing

This review demonstrates that *G. mangostana* pericarp extracts and their major xanthones, particularly α-mangostin, exhibit antimalarial activity across multiple *in vitro* assays and limited *in vivo* models. In general, α-mangostin demonstrated promising activity against both chloroquine-sensitive and -resistant *P. falciparum* strains, while non-polar fractions (e.g., dichloromethane and hexane) were frequently reported as highly active. However, *in vivo* efficacy—especially via oral administration—remains limited, likely due to pharmacokinetic constraints (e.g., poor bioavailability) rather than methodological limitations.

To better interpret these potency findings, the reported activity was evaluated in conjunction with study reliability. Studies with a lower risk of bias generally showed consistent but moderate activity, whereas several "very active" results were reported in studies with methodological limitations or incomplete reporting. This suggests that potency may be overestimated in less reliable *in vitro* studies. When focusing on more reliable evidence, α-mangostin and selected fractions remain active to promising, but fewer findings meet the "very active" category. These considerations highlight the importance of interpreting antimalarial potency in the context of study quality before drawing mechanistic or translational conclusions.

Across the included studies, SYBR Green I and pLDH assays were most frequently used; assay heterogeneity precluded pooling. The antimalarial assays used in the included studies are as follows. pLDH is frequently used to screen for antimalarial compounds targeting the asexual blood stage *of Plasmodium* sp. (D'Alessandro et al., 2013). Parasite-specific pLDH catalyzes the conversion of pyruvate to lactate to produce ATP; its inhibition disrupts energy metabolism, leading to parasite death (Sunghanghwa et al., 2024). Clinically, pLDH levels decrease within 24 hours following effective treatment (Oduola et al., 1997). The Makler et al. (1993) protocol is widely used to test the antimalarial activity of extracts, fractions, and the major xanthone MP. SYBR Green I is a fluorescent dye that binds to parasite DNA to measure *Plasmodium* growth within erythrocytes (Campo et al., 2011). Its fluorescence, measured by a plate reader, increases upon binding to double-stranded DNA, enabling estimation of parasite growth kinetics (Hawadak et al., 2023). This assay is simple, rapid, cost-effective, and suitable for routine monitoring (Rason et al., 2008).

PCA was first introduced to test the sensitivity of antimalarial drugs, particularly chloroquine (Rieckmann et al., 1978). This assay uses Giemsa stain to observe the inhibition of *Plasmodium sp*. trophozoite maturation into schizonts (Rieckmann et al., 1978). The HIA is based on the fact that *Plasmodium* spp. cannot synthesize purines de novo and must therefore acquire these metabolic precursors from external sources for growth. The addition of the radiolabeled purine [^{3}H] hypoxanthine allows parasite proliferation to be measured using a scintillation counter (Arnold et al., 2016). The addition of antimalarial compounds reduces the number of infected erythrocytes, thereby reducing the amount of detected radioisotope (Chulay et al., 1983). The HIA protocol developed by Desjardins et al. (1979) is widely used to evaluate the antimalarial activity of MP extracts, fractions, and major xanthone compounds.

Flow cytometry enables rapid detection of *Plasmodium* sp.-infected red blood cells by measuring the fluorescence of a DNA-binding dye. Compared to other dyes, YOYO-1 offers higher sensitivity and lower variability (Hirons et al., 1994).

Table 1. Study characteristics for each article.

No.	Title	Article citation	Country	Study design	Assay name	Compound/ extract/fraction	Source	Plant	Subject/target assay	Test activity (IC_{50})	Comparator or positive control	Control activity (IC_{50})	Cytotoxicity assay	Cytotoxicity (CC_{50}) of sample	SI
1	Cytotoxic and antimalarial prenylated xanthones from *Cratoxylum cochinchinese*	(Laphookhieo et al., 2006)	Thailand	*In vitro*	HIA	α-MG	Isolation	Roots of *Cratoxylum cochinchinense*	*Pf strain K1*	>48,700 µM	Dihydroartemisinin	0.0045 µM	Human lung cancer cell line (NCI-H187)	5.8 µM	NA
2	Antimalarial and vasorelaxant constituents of the leaves of *Allanblackia monticola* (*Guttiferae*)	(Azebaze et al., 2007)	Central Africa	*In vitro*	HIA	α-MG	Isolation	Leaves of *Allanblackia monticola*	*Pf strain FcM29*	0.6 ± 2.0 µM	Chloroquine	0.57 µM	Human melanoma cell	6.0 µM	10
									Pf strain F32	0.05 ± 7.7 µM		0.036 µM			120
						1,7-dihydroxy-3-methoxy-2-(3-methylnut-2-enyl)xanthone			*Pf strain FcM29*	1.5 ± 8.0 µM	Atovaquone	0.0003 µM		6.3 µM	4.2
									Pf strain F32	3.8 ± 6.5 µM		0.0008 µM			1.66
3	Endodesmiadiol, a friedelane triterpenoid, and other antiplasmodial compounds from *Endodesmia calophylloides*	(Ngouamegne et al., 2008)	Central Africa	*In vitro*	Flow Cytometry assay	8-deoxygartanin	Isolation	Stem bark of *Endodesmia calophyloides*	*Pf strain W2*	11.8 ± 0.4 µM	Chloroquine phosphate	NR	Erythrocyte susceptibility assay: >20 mM	NR	NR
4	α-MG Effect on inhibition of development stages and globin accumulation against *Plasmodium falciparum*	(Kuncoro et al., 2018)	Indonesia	*In vitro*	Globin accumulation assay	α-MG	Isolation	Stem bark of *Garcinia tetrandra* Pierre	Cysteine proteases	NA	E-64 (MW: 357.4 g/mol)	NA	NA	NA	NA
5	*In vitro* antiplasmodial and chloroquine resistance reversal effects of MG	(Ibraheem et al., 2020)	Malaysia	*In vitro* (isobologram study)	SYBR Green I assay	α-MG	Synthetic	NA	*Pf strain 3D7*	9.4 ±1.03 µM	Chloroquine	0.021 ± 0.002 µM	Vero cells	200.6 ± 11.3 µM	21.3
									Pf strain K1	9.7 ± 0.93 µM		0.265 ± 0.05 µM			20.6

Table 1. Study characteristics for each article (continued...)

No.	Title	Article citation	Country	Study design	Assay name	Compound/ extract/fraction	Source	Plant	Subject/target assay	Test activity (IC_{50})	Comparator or positive control	Control activity (IC_{50})	Cytotoxicity assay	Cytotoxicity (CC_{50}) of sample	SI
6	*In vivo* antimalarial activity of α-MG and the new xanthone δ-MG	(Upegui et al., 2015)	Colombia	*In vitro*	HIA	α-MG	Milling and isolation	MP	*Pf strain 3D7*	36.10 ± 4.9 µM	Chloroquine diphosphate	0.04 ± 0.03 µM	U937 promonocytic cell line	130.6 ± 19.7 mM	3.6
									Pf strain FCR3	0.20 ± 0.01 µM		0.28 ± 0.09 µM			653.0
						γ-MG			*Pf strain 3D7*	12.40 ± 1.0 µM		0.04 ± 0.03 µM		262.6 ± 40.2 mM	21.1
									Pf strain FCR3	>121.20 ± 1.0 µM		0.28 ± 0.09 µM			<2.2
						90% EE			*Pf strain 3D7*	11.40 ± 1.8 µg/mL		0.021 µg/mL		190.6 ± 13.0 mM	16.7
									Pf strain FCR3	0.34 ± 0.07 µg/mL		0.144 µg/mL			560.6
				in vivo	Rane's test	α-MG oral (100 mg/kg/day for 7 days)			*P. berghei* ANKA- - infected BALB/c mice	26.9%	Chloroquine diphosphate (15 mg/kg/day for 4 days)	100	NA	NA	NA
						α-MG ip (100 mg/kg/day for 7 days)				80.7%					NA
						γ-MG oral (100 mg/kg/day for 7 days)				35.0%					NA
						γ-MG ip (100 mg/kg/day for 7 days)				79.9%					NA
7	*In vitro* antimalarial activity of benzophenones and xanthones from edible fruits of *Garcinia* species	(Lyles et al., 2014)	United States of America (USA)	*In vitro*	pLDH assay	α-MG	Isolation	Whole fruit of *G. mangostana*, *G. xanthochymus*, *G. livingstonei*, and *G. spicata*	*Pf strain D6*	11.40 ± 0.00 µM	Artemisinin	0.02 ± 0.01 µM	Vero cells	NA	>1.0
											Chloroquine	0.03 ± 0.01 µM			
									Pf strain W2	10.20 ± 2.00 µM	Artemisinin	0.02 ± 0.02 µM		NR	>1.1
											Chloroquine	0.24 ± 0.04 µM			
8	Prenylated xanthones as potential antimalarial substances	(Mahabusarakam et al., 2006)	Thailand	*In vitro*	HIA	α-MG	Synthetic	NA	*Pf strain K1*	17 ± 1 µM	Chloroquine	0.59 ± 0.02 µM	NR	NA	NA

Table 1. Study characteristics for each article (continued...)

No.	Title	Article citation	Country	Study design	Assay name	Compound/ extract/fraction	Source	Plant	Subject/target assay	Test activity (IC_{50})	Comparator or positive control	Control activity (IC_{50})	Cytotoxicity assay	Cytotoxicity (CC_{50}) of sample	SI
9	Antimalarial constituents from the fruit pericarp of *Pentadesma butyracea*	(Lenta et al., 2011)	Central Africa	*In vitro*	HIA	α-MG	Isolation	Fruit pericarp of *Pentadesma butyracea*	*Pf strain W2*	2.77 µM	Chloroquine	0.11 µM	Erythrocyte susceptibility assay: >1 mg/mL	NR	NA
						Garcinone E				0.41 µM					
10	The *in vitro* antimalarial interaction of 9-hydroxycalabaxanthone and alpha-MG with mefloquine-artesunate	(Chaijaroenkul & Na-Bangchang, 2015)	Thailand	*In vitro*	SYBR Green I assay	α-MG	Synthetic	NA	*Pf strain 3D7*	17.9 µM	Artesunate	0.001 µM	NR	NA	NA
											Mefloquine	0.0133 µM			
									Pf strain K1	9.7 µM	Artesunate	0.0017 µM			
											Mefloquine	0.0071 µM			
11	Phytochemical screening and antimalarial activity of some plants traditionally used in Indonesia	(Abdillah et al., 2015)	Indonesia	*In vitro*	PCA	ME ip (100 mg/kg/day for 7 days)	Maceration	MP	*Pf strain 3D7*	0.235 ± 0.120 µg/mL	NR	NR	NR	NR	NR
				In vivo	Rane's test				*P. berghei*-infected DDY mice	≈ 15%			NA	NA	NA
12	Ball milling extraction as a green and efficient approach for the extraction of sixteen xanthone-type QR-2 and PTP1B inhibitors from *Garcinia mangostana* L. pericarp	(Li et al., 2023)	China	*In vitro*	Quinone reductase inhibitor assay	EE	BME + HRE	MP	Quinone reductase	5.26 ± 0.34 µM	Melatonin	NR	NR	NR	NR
13	Inhibitory activities of pomegranate and mangosteen extracts and their major constituents against *Plasmodium falciparum* lactate dehydrogenase	(Sunghanghwa et al., 2024)	Thailand	*In vitro* (isobolomic study)	pLDH assay	α-MG	Synthetic	NA	*Pf strain K1*	6.29 ± 1.12 µM	Artesunate	0.0052 ± 0.00 µM	Hep G2 cells	92.7 ± 3.0 µM	14.75
						γ-MG		NA		12.33 ± 2.14 µM				91.8 ± 0.43 µM	7.44
						70% EE	MAE	MP		3.44 ± 0.37 µg/mL		0.002 ± 0.00 µg/mL	NR	NA	NA

Table 1. Study characteristics for each article (continued...)

No.	Title	Article citation	Country	Study design	Assay name	Compound/ extract/fraction	Source	Plant	Subject/target assay	Test activity (IC_{50})	Comparator or positive control	Control activity (IC_{50})	Cytotoxicity assay	Cytotoxicity (CC_{50}) of sample	SI
14	Antimalarial activities of medicinal plants and herbal- -based formulations used in Thai traditional medicine	(Thiengsusuk et al., 2013)	Thailand	*In vitro*	SYBR Green I assay	95% EE	Maceration	MP	*Pf strain K1*	3.6 µg/mL	Artesunate	0.00073 µg/mL	Normal human epithelial cell	67.3 µg/mL	18.7
											Mefloquine	0.004 µg/mL			
											Chloroquine	0.066 µg/mL			
									Pf strain 3D7	11.8 µg/mL	Artesunate	0.00081 µg/mL			5.7
											Mefloquine	0.009 µg/mL			
											Chloroquine	0.005 µg/mL			
15	Metabolite footprinting of *Plasmodium falciparum* following exposure to *Garcinia mangostana* Linn. crude extract	(Chaijaroenkul et al., 2014a)	Thailand	*In vitro* (metabolite footprinting)	SYBR Green I assay	α-MG	Synthetic	NA	*Pf strain 3D7*	7.3 µg/mL	Artesunate	0.4 µg/mL	NR	NA	NA
									Pf strain K1	5.0 µg/mL		0.7 µg/mL		NR	NR
						Gartanin			*Pf strain 3D7*	9.2 µg/mL	Mefloquine	5.0 µg/mL		NR	NR
									Pf strain K1	6.8 µg/mL		2.7 µg/mL		NR	NA
						EE	Maceration	MP	*Pf strain 3D7*	12.6 µg/mL	Chloroquine	0.0034 µg/mL		NR	NR
									Pf strain K1	4.5 µg/mL		0.0402 µg/mL		NR	NR
16	Phytochemical, antimicrobial, and antiprotozoal evaluation of *Garcinia mangostana* pericarp and α-MG, its major xanthone derivative	(Al-Massarani et al., 2013)	Saudi Arabia	*In vitro*	pLDH assay	α-MG	Isolation	MP	*Pf strain K1*	2.2 µM	Chloroquine	NR	MRC-5 fibroblast cell line	7.5 µM	3.4
						Dichloromethane fraction of 70% EEs	Maceration			2.7 µg/mL				9.4 µg/mL	3.5
						ethyl acetate fraction of 70% EEs				40.3 µg/mL					>1.6

Table 1. Study characteristics for each article (continued...)

No.	Title	Article citation	Country	Study design	Assay name	Compound/ extract/fraction	Source	Plant	Subject/target assay	Test activity (IC_{50})	Comparator or positive control	Control activity (IC_{50})	Cytotoxicity assay	Cytotoxicity (CC_{50}) of sample	SI
17	Antimalarial activity and toxicity of *Garcinia mangostana* Linn.	(Bunyong et al., 2014)	Thailand	*In vitro*	SYBR Green I assay	96% EE	Maceration	MP	*Pf strain 3D7*	11.12 µg/mL	Artesunate	0.000807 µg/mL	Normal human epithelial cell	67.3 µg/mL	6.04
											Chloroquine	0.00336 µg/mL			
									Pf strain K1	7.54 µg/mL	Artesunate	0.000734 µg/mL			8.9
											Chloroquine	0.0412 µg/mL			
				In vivo	Rane's test	96% EE po (250 mg/kg/day for 4 days)			*P. berghei* ANKA-infected BALB/c mice	10.5%	Chloroquine oral (10 mg/kg/day for 4 days)	100%	NA	NA	NA
18	Proteomics analysis of antimalarial targets of *Garcinia mangostana* Linn.	(Chaijaroenkul et al., 2014b)	Thailand	*In vitro* (proteomic study)	SYBR Green I assay	EE	Maceration	MP	*Pf strain 3D7*	NA	NA	NA	NA	NA	NA
19	Antimalarial activity of *Garcinia mangostana* L. rind and its synergistic effect with artemisinin *in vitro*	(Tjahjani, 2017)	Indonesia	*In vitro*	PCA	96% EE	Maceration	MP	*Pf strain 3D7*	0.415 µg/mL	Artemisinin	NR	NR	NA	NA
						Hexane fractions				0.12 µg/mL				NR	NR
						Ethyl acetate fractions				1–10 µg/mL				NR	NR
						Butanol fractions				1,153.028 µg/mL				NA	NR
						Water fractions				No activity				NR	NR
20	Ethyl acetate fraction of *Garcinia mangostana* L. rind studied as antimalarial and antioxidant in *Plasmodium berghei*-inoculated mice	(Tjahjani et al., 2019)	Indonesia	*In vivo*	Rane's test	Ethyl acetate fraction of 96% EE, oral (100 mg/kg/day for 4 days)	Maceration	MP	*P. berghei*-infected DDY mice	81%	Artemisinin (50 mg/kg/day for 4 days)	98%	NA	NA	NA

α-MG: α-Mangostin; γ-MG: γ-Mangostin; EE: Ethanolic extract; MP: Mangosteen pericarp; NA: Not available; NR: No reported; SI: Selectivity index.

Table 2. Study bias risk for each article*.

Article reference	Parameter	
	Reporting quality (%)	Methodological quality (%)
(Laphookhieo et al., 2006)	35.71	30.00
(Azebaze et al., 2007)	63.04	63.33
(Ngouamegne et al., 2008)	88.64	100.00
(Kuncoro et al., 2018)	57.14	70.00
(Ibraheem et al., 2020)	95.24	100.00
(Upegui et al., 2015)	84.09	90.00
(Lyles et al., 2014)	78.57	93.33
(Mahabusarakam et al., 2006)	81.82	93.33
(Lenta et al., 2011)	42.86	40.00
(Chaijaroenkul & Na-Bangchang, 2015)	65.91	66.67
(Abdillah et al., 2015)	75.00	76.67
(Li et al., 2023)	69.05	76.67
(Sunghanghwa et al., 2024)	90.48	93.33
(Thiengsusuk et al., 2013)	83.36	100.00
(Chaijaroenkul et al., 2014a)	75.00	83.33
(Al-Massarani et al., 2013)	78.57	83.33
(Bunyong et al., 2014)	88.64	96.67
(Chaijaroenkul et al., 2014b)	77.27	70.00
(Tjahjani, 2017)	69.05	66.67

*These results were obtained from data processing performed in Microsoft Excel downloaded from the SciRAP web platform (www.scirap.org), as listed in Annex 4.

The HPIA is based on the inhibition of heme detoxification into the inert pigment hemozoin by *Plasmodium* parasites (Basilico et al., 1998). Heme damages the parasite's membrane, prompting the parasite to convert it into hemozoin (Villiers & Egan, 2021). An antimalarial compound is active if it can prevent the formation of hemozoin (Tekwani & Walker, 2005). This assay can be used for rapid antimalarial screening of numerous samples of extracts, fractions, or isolates because it is simple, fast, and inexpensive (Ncokazi & Egan, 2005; Xuan Trang et al., 2006).

All *in vivo* studies evaluating the antimalarial activity of MP extracts used the Rane test (Abdillah et al., 2015; Bunyong et al., 2014; Tjahjani, 2017; Upegui et al., 2015). The Rane test evaluates the curative effects of extracts/compounds on mice infected with *P. berghei* ANKA. Following infection with 1×10^7 parasitized erythrocytes, treatment was administered orally for three days starting 72 hours after infection. Parasitemia was monitored using Giemsa-stained blood smears, and the percentage of suppression was used to evaluate efficacy, while the mean survival time (MST) was used to assess overall survival. Packed cell volume (PCV), rectal temperature, and body weight were measured to assess the protection provided by the extract/compound against malaria-induced pathology (Ryley & Peters, 1970).

Antimalarial activity of xanthone compounds

MPME contains saponins, alkaloids, flavonoids, triterpenoids, tannins, and polyphenols (Qian et al., 2021). MP is rich in functional phenolic compounds, including xanthones, benzophenones, tannins, flavonoids, and anthocyanins, with approximately 2 g of pure xanthone extract being extractable from 1 kg of fruit peel (Yuvanatemiya et al., 2022). This study specifically focuses on evaluating the antimalarial activity of the dominant xanthones in mangosteen peel, namely α-MG, γ-MG, gartanin, 8-deoxygartanin, garcinone E, 1,7-dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone, and 1,3,7-trihydroxy-2,8-di-(3-methylbut-2-enyl) (Wittenauer et al., 2012).

The Pf3D7 and K1 strains are most commonly used as model systems for testing the antimalarial activity of xanthone compounds. The 3D7 strain is a chloroquine-sensitive parasite, characterized by a relatively low IC_{90} value (Ursing et al., 2022). Compared to the K1 strain, 3D7 is more susceptible to chloroquine due to three mutations that reduce glutathione reductase

(GR) activity by up to fivefold, thereby impairing the parasite's ability to neutralize oxidative stress (Gilberger et al., 2000). Other *Pf* strains mentioned in the article include F32 and D6, classified as chloroquine-susceptible, and FCR3, K1, and FcM29, classified as chloroquine-resistant (Nogueira & Lopes, 2011). *P. berghei* is also commonly used in *in vivo* studies because it does not cause malaria in humans and infects only rodents (Matz & Kooij, 2015).

The major xanthones found in the fruit peel and bark are α- and γ-MG (Rizaldy et al., 2021). α-MG is particularly abundant in the fruit peel, with concentrations reaching 121.01 mg/g of dried fruit peel (Ong et al., 2020). The heatmap indicates that α-MG exhibits consistent activity against the K1 strain across all assay methods (Fig. 2). Higher activity was observed in the HIA for the F32 and FcM29 strains, while LDH-based activity was more pronounced in D6 and W2. Responses in the SYBR Green I method were limited to a few strains. These variations between methods suggest potential differences in biological targets, indicating possible multiple pathways for α-MG. The activity observed in both chloroquine-sensitive and -resistant strains demonstrates the broad-spectrum potential of α-MG.

Differences in assay methods must be considered when interpreting the antimalarial activity of α-MG. Variability in antimalarial activity may also be due to differences in sample sources, as well as in assay methods and parasite strains used. α-MG from different sources, tested using the same parasite strain, showed that α-MG isolated from the roots *of Cratoxylum cochinchinense* exhibited no activity (Mahabusarakam et al., 2006). In contrast, its synthetic version demonstrated promising antimalarial activity (Laphookhieo et al., 2006). These differences may be due to variations in compound purity. Contaminants in natural isolates, such as solvents, buffers, and other chemicals used during extraction and testing, can interfere with antimalarial efficacy (Naidu et al., 2018).

Rane's test was also conducted to evaluate the *in vivo* antimalarial activity of α-MG isolated from MP (Upegui et al., 2015). Administration of the isolate at 100 mg/kg/day for 7 days resulted in low oral activity but high intraperitoneal activity. This difference is attributed to variations in the bioavailability of α-MG between the two administration routes. Consistent with this, oral administration of α-MG in mice demonstrated very low bioavailability, only 0.771% (Choi et al., 2014). This low bioavailability is primarily due to its low water solubility (Meylina et al., 2021) and its susceptibility to extensive first-pass hepatic and intestinal metabolism (Choi et al., 2014).

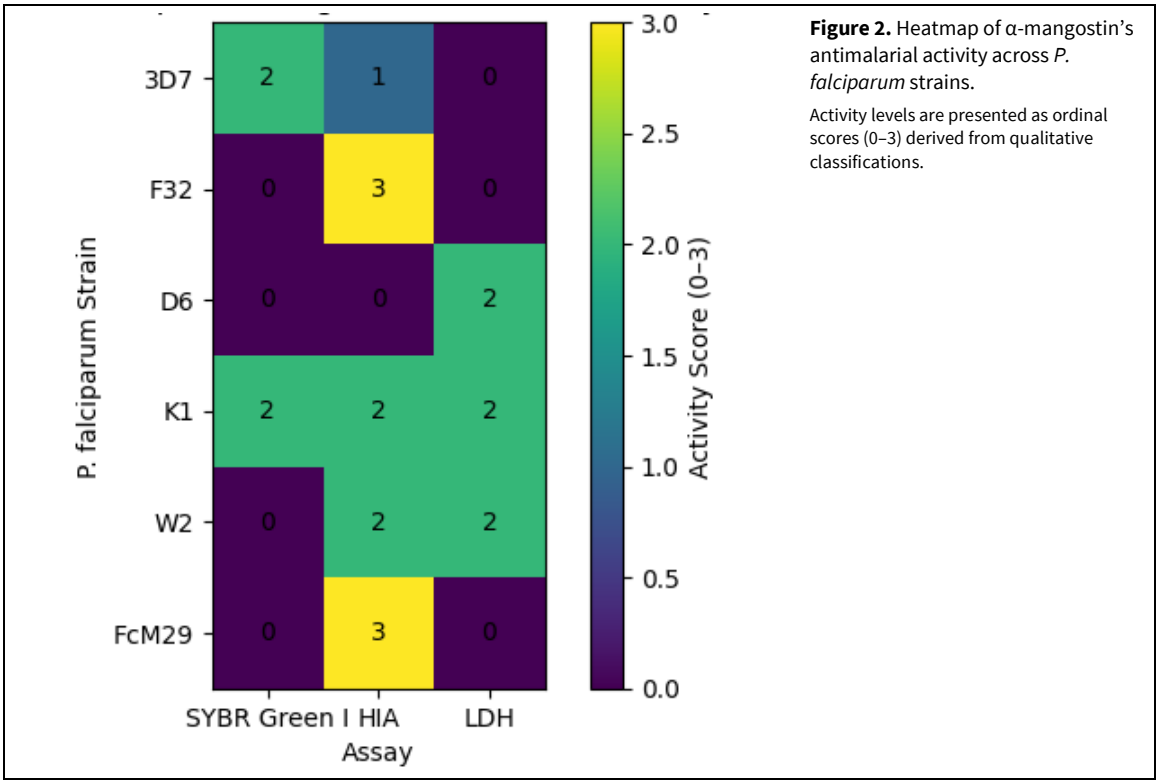


Figure 2. Heatmap of α-mangostin's antimalarial activity across *P. falciparum* strains.

Activity levels are presented as ordinal scores (0–3) derived from qualitative classifications.

Two potential mechanisms of antimalarial action have been proposed for α-MG (Ibraheem et al., 2020; Kuncoro et al., 2018). Inhibition of the cysteine protease (falcipain) during the trophozoite stage is thought to disrupt hemoglobin degradation, particularly of the globin fraction, leading to accumulation of undegraded globin in the parasite's food vacuoles (Rosenthal et al., 1991). α-MG is thought to inhibit cysteine proteases, as evidenced by swelling of food vacuoles and parasite cell death (Kuncoro et al., 2018). α-MG also inhibits the conversion of free heme to hemozoin, with an IC_{50} value of 4.2 µM (Ibraheem et al., 2020). Free heme damages the parasite's membrane, thereby reducing parasitemia (Villiers & Egan, 2021).

Although its potency is still lower than that of standard antimalarial drugs such as chloroquine, α-MG shows potential as a lead compound for further development. This potential can be enhanced through structural modifications, improved formulations, or combinations with other agents. Additionally, α-MG offers favorable safety characteristics. This compound has been shown to be safe, with reported LD_{50} values ranging from 6,000 to 15,480 mg/kg body weight (Setyawati et al., 2023). Furthermore, α-MG does not cause toxic effects on biochemical parameters, body weight, and organs, or histopathological markers (Eisvand et al., 2022).

γ-MG demonstrated antimalarial activity against the Pf3D7 strain (promising) and the FCR3 strain (inactive) based on the HIA assay (Upegui et al., 2015). It also showed promising activity against the K1 strain in the pLDH assay (Sunghanghwa et al., 2024). In the Rane test at a dose of 100 mg/kg/day for 7 days, it showed weak activity via oral administration and moderate activity via intraperitoneal injection (Upegui et al., 2015). Comparative studies confirmed that α-MG exhibits superior antimalarial efficacy both *in vitro* and *in vivo* (Sunghanghwa et al., 2024; Upegui et al., 2015). The compound γ-MG exhibits better QR-2 inhibitory activity than other xanthone compounds [8-deoxygartanin; 1,3,7-trihydroxy-2,8-di(3-methylbut-2-enyl)xanthone; α-MG; and garcinone E], all of which exhibit better antioxidant activity than the melatonin control (Liang et al., 2020).

Gartanin exhibited moderate and promising antimalarial activity against the Pf3D7 and K1 strains, respectively, when tested using the SYBR Green I assay. In a comparative study, α-MG demonstrated superior activity compared to gartanin (Chaijaroenkul et al., 2014a). On the other hand, garcinone E exhibited strong activity against the W2 strain in the HIA and was more effective than α-MG, which showed only promising activity (Lenta et al., 2011).

Other xanthone derivatives also demonstrate antimalarial potential. 8-Deoxygartanin exhibited promising activity against strain W2 as measured by flow cytometry (Ngouamegne et al., 2008). Similarly, 1,3,7-trihydroxy-2,8-di-(3-methylbut-2-enyl)xanthone demonstrated promising activity against strains FcM29 and F32 after 72 hours of exposure in the HIA assay, although it was less active than α-MG (Azebaze et al., 2007).

Antimalarial activity of MP extracts and fractions

The antimalarial activity of mangosteen peel extract and its fractions is summarized in Table 3. Differences in the antimalarial efficacy of MP extract can be observed due to variations in extraction methods and solvents. Most studies used maceration with ethanol as the primary extraction technique, yielding extracts with varying levels of antimalarial activity depending on solvent concentration and assay type.

One proposed mechanism of the extract's antimalarial activity, as suggested by proteomic studies, is the disruption of glycolysis when administered at an IC_{50} and increased cell damage due to enhanced proteasome activity when administered at an IC_{90} (Chaijaroenkul et al., 2014b). Another mechanism is EE inhibition of quinone reductase, with an IC_{50} of 5.26 ± 0.34 µM (Li et al., 2023). QR-2 catalyzes the reduction of menadione (a reactive quinone) into the relatively stable hydroquinone form (Choi et al., 2011). Inhibition of QR-2 increases quinone redox cycling, thereby increasing ROS formation and disrupting *Plasmodium* metabolism (Cassagnes et al., 2017). Evidence of MP extract activity indicates a polypharmacological rather than a single-target mechanism, as demonstrated by metabolite footprinting studies, which show disruption of glycolysis, amino acid metabolism, and redox balance, thereby inhibiting energy production and parasite growth (Chaijaroenkul et al., 2014a).

90%EE demonstrated active antimalarial activity against *Pf* strain 3D7 and very active activity against FCR3 based on the HIA assay (Upegui et al., 2015). Similar results were observed for 95–96% EE tested using SYBR Green I, showing activity against 3D7 and very strong activity against the K1 strain (Bunyong et al., 2014; Chaijaroenkul et al., 2014a; Thiengsusuk et al., 2013). 70% EE tested with the pLDH assay also showed highly active results against the K1 strain (Sunghanghwa et al., 2024). This indicates that K1 is more susceptible to EE than 3D7, and the difference in ethanol concentration between 70% and 96% does not significantly alter efficacy (Bunyong et al., 2014; Chaijaroenkul et al., 2014a; Sunghanghwa et al., 2024; Thiengsusuk et al., 2013).

Table 3. Antimalarial activity of MP extracts and fractions.

Article citation	Study design	Assay	Extracts/fraction	Source	Subject/target assay	Test activity (IC_{50})	Positive control	Control activity (IC_{50})	Interpretation of test sample
(Upegui et al., 2015)	*In vitro*	HIA	90% EE	Milling	*Pf strain 3D7*	11.40 ± 1.8 µg/mL	Chloroquine diphosphate	0.021 µg/mL	Active
					Pf strain FCR3	0.34 ± 0.07 µg/mL		0.144 µg/mL	Very active
(Abdillah et al., 2015)	*In vitro*	PCA	ME	Maceration	*Pf strain 3D7*	0.235 ± 0.120 µg/mL	NR	NR	Very active
	In vivo	Rane's test	ME ip (100 mg/kg/day for 7 days)		*P. berghei*-infected DDY mice	~15%			low
(Sunghanghwa et al., 2024)	*In vitro*	pLDH	70% EE	MAE	*Pf strain K1*	3.44 ± 0.37 µg/mL	Artesunate	0.002 ± 0.00 µg/mL	Very active
(Thiengsusuk et al., 2013)	*In vitro*	SYBR Green I	95% EE	Maceration	*Pf strain K1*	3.6 µg/mL	Artesunate	0.00073 µg/mL	Very active
							Mefloquine	0.004 µg/mL	
							Chloroquine	0.066 µg/mL	
					Pf strain 3D7	11.8 µg/mL	Artesunate	0.00081 µg/mL	Active
							Mefloquine	0.009 µg/mL	
							Chloroquine	0.005 µg/mL	
(Chaijaroenkul, et al., 2014a)	*In vitro*	SYBR Green I	EE	Maceration	*Pf strain 3D7*	12.6 µg/mL	Chloroquine	0.0034 µg/mL	Active
					Pf strain K1	4.5 µg/mL		0.0402 µg/mL	Very active
(Al-Massarani et al., 2013)	*In vitro*	pLDH	Dichloromethane fraction of 70% EE	Maceration	*Pf strain K1*	2.7 µg/mL	Chloroquine	NR	Very active
			Ethyl acetate fraction of 70% EE			40.3 µg/mL			Active
(Bunyong et al., 2014)	*In vitro*	SYBR Green I	96% ethanolic extract	Maceration	*Pf strain 3D7*	11.12 µg/mL	Artesunate	0.000807 µg/mL	Active
							Chloroquine	0.00336 µg/mL	
					Pf strain K1	7.54 µg/mL	Artesunate	0.000734 µg/mL	Active
							Chloroquine	0.0412 µg/mL	
	In vivo	Rane's test	96% EE po (250 mg/kg/day for 4 days)		*P. berghei* ANKA-infected BALB/c mice	10.5%	Chloroquine oral (10 mg/kg/day for 4 days)	100%	Low
(Tjahjani, 2017)	*In vitro*	PCA	96% EE	Maceration	*Pf strain 3D7*	0.415 µg/mL	Artemisinin	NR	Very active
			Hexane fractions			0.12 µg/mL			Very active
			Ethyl acetate fractions			1–10 µg/mL			Active
			Butanol fractions			1,153.028 µg/mL			Inactive
			Water fractions			No activity			Inactive
(Tjahjani et al., 2019)	*In vivo*	Rane's test	Ethyl acetate fraction of 96% EE, oral (100 mg/kg/day for 4 days)	Maceration	*P. berghei*-infected DDY mice	81%	Artemisinin (50 mg/kg/day for 4 days)	98%	high

The type of fractionation solvent plays a crucial role in the antimalarial activity of the extract fractions. The dichloromethane fraction of the 70% EE exhibits highly active antimalarial activity, while the ethyl acetate fraction is only active (Al-Massarani et al., 2013). Another study showed that the hexane fraction of the 96% EE exhibits strong antimalarial activity, while the ethyl acetate fraction is active, and the butanol and water fractions are inactive (Tjahjani, 2017). The dichloromethane and hexane fractions exhibited the highest *in vitro* antimalarial activity (Tjahjani, 2017), suggesting that nonpolar compounds other than xanthones may play a significant role in this effect.

EE exhibits active to highly active *in vitro* antimalarial activity (Bunyong et al., 2014; Chaijaroenkul et al., 2014a; Sunghanghwa et al., 2024; Thiengsusuk et al., 2013), while ME exhibited very active activity (Abdillah et al., 2015). This is attributed to differences in solvents, which yield varying concentrations of antimalarial active compounds (including xanthones) (Kusmayadi et al., 2019). Ethyl acetate is known to extract the highest amount of α-MG (75.66 mg/g dry weight), followed by ethanol (Ghasemzadeh et al., 2018). These results are supported by other studies that found ethyl acetate and ethanol to be the most effective solvents for isolating α-MG from the pericarp (Bundeesomchok et al., 2016).

The extraction method contributes little to variations in biological activity. Techniques used in antimalarial studies include grinding (Upegui et al., 2015), MAE (Sunghanghwa et al., 2024), and maceration (Al-Massarani et al., 2013; Bunyong et al., 2014; Chaijaroenkul et al., 2014a; Thiengsusuk et al., 2013; Tjahjani, 2017). The results indicate that nearly all fractions and extracts from various extraction methods are active, although none of the extracts or fractions exhibit activity superior to that of the single compound chloroquine.

Rane's test evaluates antimalarial efficacy in mice infected with *P. berghei*. Treatment with EE and the ethyl acetate fraction significantly reduced parasitemia in DDY mice compared to the untreated control group (Abdillah et al., 2015; Tjahjani et al., 2019). A similar effect was observed in BALB/c mice, where oral administration of EE provided 10.5% inhibition, which was still lower than the 100% inhibition achieved by chloroquine (Bunyong et al., 2014). The lower *in vivo* efficacy is likely due to the poor oral bioavailability of α-MG, which is caused by low solubility and extensive first-pass metabolism (Choi et al., 2014). Importantly, all included *in vivo* studies met the criteria for 'reliable without restrictions' based on ToxRTool assessment, supporting the internal validity of these findings; therefore, the observed limitations in efficacy should be interpreted as biological or pharmacokinetic constraints rather than methodological weaknesses.

Combination of extracts or compounds with conventional antimalarial drugs

The combination of α-MG with mefloquine showed a neutral interaction (CI = 1) against the PfK1 and 3D7 strains, while the combination with artesunate was antagonistic (CI > 1) (Chaijaroenkul & Na-Bangchang, 2015). Loewe model-based synergy analysis indicates that α-MG exhibits an additive effect (synergy score: (-10)—10) when combined with artesunate, suggesting the absence of strong synergistic effects (Sunghanghwa et al., 2024). Conversely, the combination of α-MG and chloroquine (3:7 ratio) in an isobologram study showed synergistic results (FIC = 0.5–1) by increasing parasite sensitivity, likely through α-MG's inhibition of hemozoin formation, enhanced accumulation of chloroquine in digestive vacuoles, and potential support for chloroquine-induced apoptosis (Ibraheem et al., 2020). Results from the crude extract and MP fraction combined with artemisinin showed increased antimalarial efficacy and may help prevent resistance (Tjahjani, 2017). These findings suggest the potential use of xanthone compounds or extracts to enhance the antimalarial effects (additive or synergistic) of drugs, depending on the drug combination.

Toxicity of MP extract

The toxicity studies identified in the collected articles consist of cytotoxicity studies (Annex 6). The target cells for these cytotoxicity studies were red blood cells (Lenta et al., 2011; Ngouamegne et al., 2008); Vero cells (Ibraheem et al., 2020; Lyles et al., 2014); human epithelial cells (Bunyong et al., 2014; Thiengsusuk et al., 2013); human melanoma cells (Azebaze et al., 2007); human lung cancer cell lines (Laphookhieo et al., 2006); promonocytic cell lines (Upegui et al., 2015); Hep G2 cells (Sunghanghwa et al., 2024); and MRC-5 fibroblast cell lines (Al-Massarani et al., 2013). The erythrocyte cytotoxicity study was conducted using an erythrocyte susceptibility assay to determine the minimum xenobiotic dose required to cause cell death (Cedillo-Rivera et al., 1992). This assay differs from other cytotoxicity assays, such as the MTT assay, because red blood cells lack DNA (Crupi et al., 2019). Furthermore, this assay does not measure CC_{50}, so the SI cannot be calculated for studies that report it. The cytotoxicity of red blood cells by the compounds α-MG, garcinone E, and 8-deoxygartanine is >2.43, 2.46, and 20 mM, respectively (Lenta et al., 2011; Ngouamegne et al., 2008). Xenobiotics can affect erythrocytes by damaging cell membrane integrity, thereby causing hemolysis, or by increasing intracellular Ca^{2+}, which triggers erythroptosis through cell shrinkage and exposure of

phosphatidylserine on the membrane surface (Pagano & Faggio, 2015).

The reported SI values across studies show considerable variation, ranging from low (<2) to very high (>600) (Table 1). SI values not reported directly were calculated based on the ratio of antimalarial activity to cytotoxicity. Compounds with an SI value greater than 10 are considered promising antimalarial candidates, as they demonstrate high selectivity for the parasite over host cells (Syahri et al., 2023). This indicates that the selectivity of α-MG and related compounds is highly influenced by experimental conditions, parasite strains, and assay systems. In general, α-MG exhibits moderate to high selectivity (3–21), whereas crude extracts and fractions often exhibit higher SI values. This is likely because the toxicity of extracts and fractions in previous studies showed lower results than that of single compounds (Table 1). Strain-dependent differences were also observed, with higher SI values in the FCR3 strain compared to 3D7.

Studies have consistently shown that MP extract exhibits low acute and subacute toxicity. A 95% MPEE in rats showed no significant adverse effects at a single dose of up to 5 g/kg (acute) and a repeated dose of 1 g/kg/day for 28 days (subacute) (Jujun et al., 2008). A similar study tested acute toxicity at 5 g/kg and subacute toxicity at 2 g/kg for 14 days, yielding comparable results: normal behavior, no changes in body weight or organ weight, and no deaths or signs of toxicity during the testing period (Bunyong et al., 2014). Hematological and biochemical parameters remained within normal ranges, and no macroscopic or histopathological abnormalities were found in vital organs, including the liver, kidneys, lungs, and stomach (Bunyong et al., 2014; Jujun et al., 2008). In the satellite group monitored post-treatment, no delayed toxicity was detected, supporting the safety and reversibility of the extract at the tested doses (Jujun et al., 2008). Acute toxicity testing of MPME also demonstrated low toxicity in mice (LD_{50} = 231.5 mg/kg) (Choi et al., 2014).

A single oral dose of 2,000 mg/kg of MP water extract also did not cause mortality or signs of toxicity in rats up to day 14. Subchronic testing, with daily oral doses of 10, 50, and 100 mg/kg for 90 days, also showed no treatment-related mortality or significant toxic effects. Minor changes in hematology and biochemistry remained within the normal range, and histology showed no dose-dependent abnormalities. The extract was considered safe up to 100 mg/kg/day, although further studies are recommended regarding renal effects (Chayaburakul et al., 2015).

Conversely, chronic exposure at higher doses indicated potential toxicity (Chivapat et al., 2011). Administration of 95% EE for six months at doses ≥500 mg/kg/day caused weight loss, elevated liver enzymes (ALT, AST), hepatic cell degeneration, and increased BUN and creatinine levels, indicating hepatic and renal stress. Persistent liver abnormalities in the control group indicate irreversible changes at high doses. Similarly, exposure to EE at 200–400 mg/kg/day for 6 months caused liver necrosis and renal histopathological changes, with increases in GOT, creatinine, and triglycerides—particularly in females (Mahmudah et al., 2024). These findings indicate that although the extract is safe at low doses, chronic exposure at ≥200 mg/kg/day may pose a risk to liver and kidney health.

Pharmacokinetics of the major xanthone compound in MP extract (α-MG)

The failure of most drugs in the clinical phase is attributed to inadequate pharmacokinetic profiles in humans (Parasrampuria et al., 2018). Knowledge of the pharmacokinetic profiles of xanthone compounds can be used in the selection of compounds predicted to pass clinical phase testing (Miller et al., 2005). Pharmacokinetic studies of the main xanthone compound in the extract (α-MG) have been extensively conducted previously (Table 4).

The α- and γ-MG compounds exhibit high stability in the gastrointestinal tract, with >90% remaining intact in simulated digestive fluids (Bumrungpert et al., 2009). Nevertheless, intravenous administration yields a significantly higher AUC than the oral route, indicating low bioavailability of α-MG due to limited absorption and first-pass effects. The predominant first-pass metabolism involves Phase II metabolism, specifically glucuronidation (Bumrungpert et al., 2009; Choi et al., 2014; Ramaiya et al., 2012). This low oral bioavailability aligns with findings that oral suppression of parasitemia is lower than that via the intraperitoneal route (Upegui et al., 2015). Formulation innovations or administration routes that circumvent first-pass metabolism require further investigation to enhance α-MG bioavailability.

Oil-based formulations and soft capsules can increase the AUC of α-MG compared with a vehicle mixture of water and PEG (Petiwala et al., 2014; Ramaiya et al., 2012; Zhao et al., 2016). The use of extracts can also increase the AUC of α-MG due to the presence of other compounds. Tannins can form water-soluble complexes with lipophilic compounds (Jackson & Letchford, 2016). Saponins are known to enhance the solubilization of lipophilic compounds by forming micelles (Lutgen, 2018). The absorption of α-MG is highly dependent on the dose-dependent micellization of bile salts (Bumrungpert et al., 2009).

Table 4. Pharmacokinetic studies of α-MG.

Compound / sample	Vehicle/formulation	Species (strain)	Experimental groups	Dose (mg/kg)	Route	Cmax (ng/mL)	Tmax (h)	$AUC_{0-\infty}$ (ng·h/mL)	t½ (h)	Metabolism	Clearance (L/h/kg)	References
α-MG	PEG 400: water (6:4)	Mice (ICR)	α-MG-treated group, 6 doses (n = 7–9/group)	5	IV	NA	NA	2817	3.77	NR	1.88	(Han et al., 2015)
				10		NA	NA	5667	3.65		1.88	
				20		NA	NA	10,550	2.88		2.08	
				10	po	40.3	1	130	2.95		NR	
				50		242	0.5	622	3.28		NR	
				100		709	1	1387	2.52		NR	
α-MG	PEG 400: water (6:4)	Mice (ICR)	α-MG (n = 8) and extract-treated group (n = 6)	5	IV	NA	NA	3467	3.05	Liver and intestinal tissue	1.48	(Choi et al., 2014)
α-MG (in MP extract)					IV	NA	NA	5583	1.88		0.94	
α-MG			α-MG (n=5) and extract-treated group (n = 5)	20	po	40.8	0.25	93	NR		0.181	
α-MG (in MP extract)					po	86.5	1.0	172	NR		0.128	
α-MG (in MP extract)	PEG 400: water (6:4)	Mice (ICR)	α-MG-treated group, 2 doses (n = 3 per group)	40	IV	NA	NA	96,000	2.28	NR	6.48	(Han et al., 2013)
				400	po	205	0.5	39,200	1.78	NR	NR	
α-MG	Aqueous solution containing 2% ethanol and 2% Tween 80	Rat (Sprague Dawley)	α-MG-treated group (n = 8)	2	IV	17880	0	1372	2.97	NR	1.77	(Li et al., 2011)
α-MG	Soft capsule (vegetable oil matrix)	Rat (Sprague Dawley)	α-MG-treated group, 3 doses (n = 6/group)	1.025	IV	NA	NA	100,780	11.98	NR	0.01	(Zhao et al., 2016)
					po	NR	NR	61,565	6.48		0.016	
				4.1	IV	NA	NA	218,212	14.37		0.018	
					po	NR	NR	112,426	8.55		0.036	
				16.4	IV	NA	NA	919889	16.73		0.017	
					po	NR	NR	390,982	8.71		0.042	
α-MG (in MF extract)	Suspension in cottonseed oil	Mice (C57BL/6)	α-MG-treated group (n = 32) and vehicle control group (n = 4)	36 mg/kg	PO	357	1	1216	8.2	Glucuronidation	NR	(Petiwala et al., 2014)
α-MG	Suspension in cottonseed oil	Mice (C57BL/6)	α-MG-treated group (n = 32) and vehicle control group (n = 4)	100 mg/kg	po	567	0.5	2550	5	Glucuronidation	NR	(Ramaiya et al., 2012)

This indicates that the solubilization of α-MG (via oil-based formulations or micellization) can overcome absorption barriers, thereby increasing its bioavailability. Existing research shows that increased solubility of α-MG via a self-microemulsion formulation can enhance α-MG bioavailability, as evidenced by a rise in Cmax (~11×), accelerated Tmax, and an increase in AUC up to 4.75 times (Xu et al., 2017).

The α-MG compound is rapidly absorbed, with Tmax occurring 0.25–1 hour after oral administration. Distribution at 1 hour post-administration of α-MG is predominantly found in the stomach, pancreas, and liver due to slow oral absorption; however, after 4 hours, distribution is primarily in the small intestine, ovaries, stomach, and bone (Zhao et al., 2016). The high concentration in the stomach after 1 hour is due to the high molecular weight (MW) of the α-MG compound (Winata et al., 2025). Meanwhile, the high concentration in the intestines after 4 hours is due to α-MG being extensively excreted into the intestines following first-pass metabolism in the liver and also acting as a substrate for the efflux transporter (P-gp) (Choi et al., 2014; Dechwongya et al., 2020). The clearance of α-MG is relatively rapid, ranging from 1.48 to 2.08 L/h/kg (Table 4). This rapid clearance suggests that a sustained-release formulation may provide greater biological activity.

Certainty of evidence

Given the identified methodological limitations, inconsistencies across studies, and the predominance of indirect evidence, the overall certainty of evidence is low to very low. While some findings—such as the *in vitro* activity of α-mangostin—appear consistent, confidence in their translational relevance remains limited.

Limitations

The main limitations stem from methodological weaknesses in the included studies. Most *in vitro* studies exhibit a high risk of bias due to a lack of methodological transparency. Of the four *in vivo* studies, only two were classified as reliable with limitations, while the remainder were deemed unreliable by the ToxRTool assessment. Inconsistent methods—ranging from parasite strains and testing techniques to extraction protocols—prevent quantitative synthesis via meta-analysis.

The review process itself has limitations, including the exclusion of non-English-language literature, which may have resulted in relevant omissions. Gray literature and unpublished data were not considered, which may introduce publication bias. Although double-blinding was performed using Rayyan software, there remains a risk of selection and data extraction bias due to the absence of third-party validation.

Implications

These findings suggest that MP and its xanthones have potential for development as complementary antimalarial agents. The *in vivo* activity of xanthone combinations or MP extracts with currently used antimalarial drug combinations has not yet been studied. Interactions between the two have also not been investigated. Existing studies have shown only that xanthones from MP act as substrates and inhibitors of several CYP450 isoforms (Foti et al., 2009). Further research on the interactions of xanthones from MP and their combination studies with current malaria regimens could aid in the future application of xanthones or MP in therapy.

This study indicates that the toxicity profile of xanthones or MP extract is relatively low. This supports their application in antimalarial therapy. However, the activity of xanthones from MP is still lower than that of existing standard drugs. Optimization of xanthones using a QSAR approach is feasible. Previous studies have investigated xanthone compounds and found that the presence and position of hydroxyl groups (particularly polyhydroxy groups) play a crucial role in enhancing interactions with heme, while methoxy substitutions also increase affinity for targets such as PfLDH (Chaniad et al., 2024; Riscoe et al., 2005).

MP extracts and fractions, as well as xanthones, did not exhibit strong antimalarial activity *in vivo* in the Rane assay. The Rane assay conducted did not perform cytokine profiling in malaria-infected mice. Studies indicate that α-MG can inhibit cytokine storm during dengue virus infection (Yongpitakwattana et al., 2021). MP extracts and their main compounds have been shown to inhibit inflammatory mediators *in vivo* (Yongpitakwattana et al., 2021). This demonstrates the holistic protective effects of MP extracts/xanthone compounds in malaria treatment beyond their direct action on the parasite.

CONCLUSION

Mangosteen fruit peel extract and its primary xanthones, particularly α-MG, demonstrate promising antimalarial activity in *in vitro* models. Their activity remains weak compared with standard drugs when used alone; however, several studies indicate synergistic or additive effects with certain standard drugs, suggesting they could complement existing antimalarial medications. Limitations such as low bioavailability can be addressed with appropriate formulations or routes of administration. However, the interpretation of these findings is limited by the prevalence of high-risk-of-bias studies, variability in results across studies, and the lack of direct *in vivo* evidence, resulting in an overall certainty of evidence ranging from low to very low. Therefore, although these results indicate potential, further studies are needed to support their use as an antimalarial therapy.

CONFLICT OF INTEREST

The research was conducted entirely free from any involvement in commercial, financial, or personal matters that might be perceived as a possible conflict of interest or that could have influenced the outcomes or interpretation of the study.

ACKNOWLEDGMENTS

This research was funded by the Directorate of Research and Community Service Development (DP2M), Ministry of Education, Culture, Research, and Technology of Indonesia, for its financial support through the PMDSU 2024 grant (Contract No. 2523/UN1/DITLIT/Dit-Lit/PT.01.03/2025).

GENERATIVE ARTIFICIAL INTELLIGENCE (AI)

The authors used ChatGPT (OpenAI) and DeepL Write to improve the language and readability of the manuscript. The authors reviewed and edited the content and take full responsibility for its accuracy. No images were generated or manipulated using AI tools.

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AUTHOR CONTRIBUTION:

Contribution	Winata JT	Nugroho AK	Murti YB	Nurrochmad A
Concept or idea	x	x	x	x
Design	x	x	x	x
Definition of intellectual property	x	x	x	x
Literature review	x	x		
Data extraction	x	x		
Risk of bias assessment	x	x		
Evidence synthesis	x	x	x	x
Data acquisition	x	x		
Data analysis	x	x		
Statistical analysis				
Manuscript preparation	x	x	x	x
Manuscript editing	x	x	x	x
Manuscript review	x	x	x	x

Citation Format: Winata JT, Nugroho AK, Murti YB, Nurrochmad A (2026) Antimalarial activity and toxicity of *Garcinia mangostana* L. pericarp extracts and major xanthones: A systematic review of *in vitro* and *in vivo* studies. J Pharm Pharmacogn Res 14(2): 2495. https://doi.org/10.56499/jppres

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Supplementary data

Annex 1. PRISMA 2020 item checklist. https://doi.org/10.1371/journal.pmed.1003583.t001

Section and topic	Item #	Checklist item	Location where item is reported
Title	1	Identify the report as a systematic review.	Title
Abstract	2	See the PRISMA 2020 for Abstracts checklist (Table 2).	Abstract
Introduction – Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Introduction
Introduction – Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Review methodology
Methods – Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Eligibility criteria
Methods – Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Information sources
Methods – Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Search strategy
Methods – Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Selection process
Methods – Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Data collection process
Methods – Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Data items (outcome)
Methods – Data items	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Not conducted
Methods – Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Study of risk of bias assessment
Methods – Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Not conducted
Methods – Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Eligibility for synthesis
Methods – Synthesis methods	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Preparing for synthesis
Methods – Synthesis methods	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Tabulation and graphical methods
Methods – Synthesis methods	13d	Describe any methods used to synthesise results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Statistical synthesis methods
Methods – Synthesis methods	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Not conducted
Methods – Synthesis methods	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesised results.	Not conducted

Methods – Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Not conducted
Methods – Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Not conducted
Results – Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram (see Fig. 1).	flows of the study
Results – Study selection	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Excluded studies
Results – Study characteristics	17	Cite each included study and present its characteristics.	Study characteristics
Results – Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Risk of bias in studies
Results – Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Not conducted
Results – Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Characteristics of contributing studies
Results – Results of syntheses	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Not conducted
Results – Results of syntheses	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Not conducted
Results – Results of syntheses	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesised results.	Not conducted
Results – Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Not conducted
Results – Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Not conducted
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	interpretation
Discussion	23b	Discuss any limitations of the evidence included in the review.	limitations of evidence
Discussion	23c	Discuss any limitations of the review processes used.	limitations of review process
Discussion	23d	Discuss implications of the results for practice, policy, and future research.	implications
Other information – Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Not conducted
Other information – Registration and protocol	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Not conducted
Other information – Registration and protocol	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Not conducted
Other information – Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Acknowledgment
Other information – Competing interests	26	Declare any competing interests of review authors.	Conflict of interests
Other information – Availability of data, code, and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Annex/supplementary

Annex 2. PRISMA 2020 for Abstracts checklist*. https://doi.org/10.1371/journal.pmed.1003583.t002

Section and topic	Item #	Checklist item	Reported
Title	1	Identify the report as a systematic review.	✓
Background – Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	✓
Methods – Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	✓
Methods – Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	✓
Methods – Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	✓
Methods – Synthesis of results	6	Specify the methods used to present and synthesise results.	✓
Results – Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	✓
Results – Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	✓
Discussion – Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	✓
Discussion – Interpretation	10	Provide a general interpretation of the results and important implications.	✓
Other – Funding	11	Specify the primary source of funding for the review.	X
Other – Registration	12	Provide the register name and registration number.	X

Annex 3. Search string.

Database	Query	Link	Date
Wiley Online Library	(Malaria* OR plasmodium OR antiplasmodial) AND (mangosteen OR garcinia OR xanthone OR mangostin OR gartanine)	https://bit.ly/3ELsxX1	12 February 2025
Science Direct	(Malaria OR malarial OR plasmodium OR antiplasmodial) AND (mangosteen OR garcinia OR xanthone OR mangostin OR gartanine)	https://bit.ly/4jTZVLu	
Scopus	(Malaria* OR plasmodium OR antiplasmodial) AND (mangosteen OR garcinia OR xanthone OR mangostin OR gartanine)	https://bit.ly/4aUx19O	
PubMed	(Malaria* OR plasmodium OR antiplasmodial) AND (mangosteen OR garcinia OR xanthone OR mangostin OR gartanine)	https://shorturl.at/Qjjpo	14 February 2025
Google Scholar	Antimalarial mangosteen	https://shorturl.at/3jc0M	
	Antimalarial xanthones	https://shorturl.at/L9MXL	15 February 2025

Annex 4. SciRAP *in vitro* tool version 2.1.

This tool consists of 3 sections: methodological quality, reporting quality, and relevance. You can evaluate either of these sections, or all. For each quality criterion or relevance item, choose one of the options from the drop-down menu in the "SELECTION" column (fulfilled, partially fulfilled, or not fulfilled for reporting quality criteria; fulfilled, partially fulfilled, not fulfilled, or not reported for methodological quality criteria; directly relevant, indirectly relevant, or not relevant for relevance items). If a criterion for reporting or methodological quality is not applicable for the study or evaluation, choose "REMOVE" in the "SELECTION" column. If you cannot find any information regarding a specific item in the study, choose the option "not reported" from the menu. Use the "COMMENT" column to write comments, such as explaining your evaluation of a specific criterion. Criterion 24 in the reporting quality section and criterion 16 in the methodological quality section do not have the drop-down menu and provide space to write free comments on any other aspects that can influence study quality. Guidance is available for evaluating methodological quality and relevance by scrolling over each criterion/item. The results of your evaluation are summarised below the list of criteria in graphical visualizations and a table indicating % fulfilled criteria.

Article 19

Proteomics analysis of antimalarial targets of Garcinia mangostana Linn.

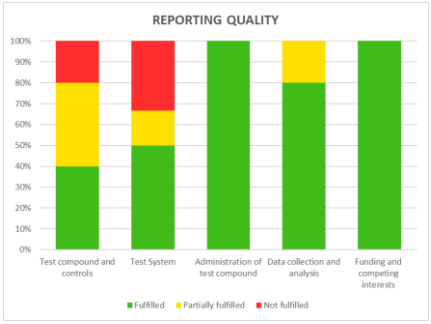
No.	REPORTING QUALITY	SELECTION	COMMENT
	Test compound and controls		
1	The chemical name or other identification, such as CAS-number, of the test compound was given.	fulfilled	
2	The purity of the test compound was stated or is traceable according to information given regarding manufacturer and lot/batch number. In case of mixtures, the composition of different constituents was stated.	partially fulfilled	
3	The solubility of the test compound was described.	partially fulfilled	
4	The solvent (vehicle) was described.	fulfilled	
5	It was stated that a solvent (vehicle) control was included.	not fulfilled	
	Test system		
6	The test system (e.g., cell line / cells/ tissue / organ / embryo / sub-cellular fractions) was described.	fulfilled	
7	The source of the test system was stated.	fulfilled	
8	The metabolic competence, i.e., competence of the test system to metabolize the test compound into an active metabolite was described.	not fulfilled	
9	The number of cell passages of the cell line used, was stated. (Remove this criterion if the study was not conducted in a cell line.)	REMOVE	
10	Composition of media was described, including use of serum, antibiotics, etc.	fulfilled	
11	Incubation temperature, humidity, and CO2 concentration were described.	partially fulfilled	
12	Measures taken for avoiding or screening for contamination by mycoplasma, bacteria, fungi and virus were described.	not fulfilled	
	Administration of test compound		
13	The administered dose levels or concentrations were stated.	fulfilled	
14	Cell density or number of cells used during treatment was described. (Remove this criterion if the study was not conducted in a cell line.)	fulfilled	
15	The duration of treatment was stated.	fulfilled	
16	The number of replicates per dose level/concentration or the number of times the experiment was repeated was stated.	fulfilled	
	Data collection and analysis		
17	The tests and/or analytical methods used were sufficiently described to allow for evaluation of reliability of results.	fulfilled	
18	The time points for data collection were stated.	fulfilled	
19	It was stated that the effect of the test compound on cytotoxicity was measured.	fulfilled	
20	All results were clearly presented.	fulfilled	
21	The statistical methods and software used were described.	partially fulfilled	
	Funding and competing interests		
22	The funding sources for the study were stated.	fulfilled	
23	Any competing interests were disclosed or it was explicitly stated that the authors did not have any competing interests.	fulfilled	
	Other		
24	Was all information that is indispensable for evaluating the reliability of data given? This includes information on the test compound and controls, test system, study design or study performance.		

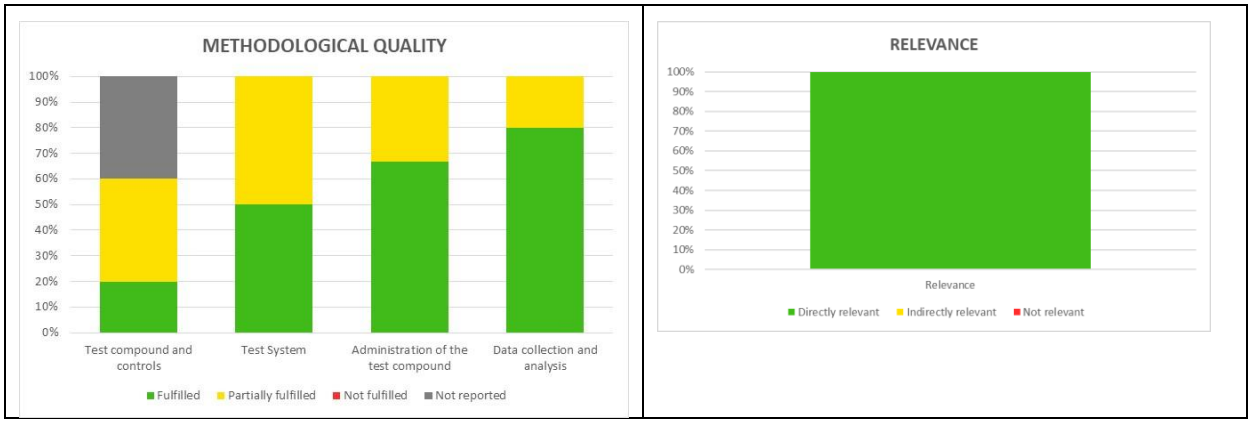
	METHODOLOGICAL QUALITY	SELECTION	COMMENT
	Test compound and controls		
1	The test compound or mixture was unlikely to contain any impurities that may significantly have affected the results of the study.	partially fulfilled	
2	It was likely that the test compound was soluble at the concentrations used.	partially fulfilled	

3	An appropriate solvent (vehicle) was used that is not expected to interfere with the results of the study at the concentration used.	fulfilled
4	A solvent (vehicle) control was included.	not reported
5	An appropriate positive control was included, and the expected result was observed from this treatment.	not reported
	Test system	
6	A reliable and sensitive test system (e.g., cell line / cells/ tissue / organ / embryo / sub-cellular fractions) with metabolic competence, if relevant, was used for investigating the test compound and endpoints.	fulfilled
7	Conditions for cultivation and/or maintenance of the cell line / cells / tissue / organ /embryo / sub-cellular fractions (incubation temperature, humidity, CO2 concentration, media used, number of cell passages, control of contamination) were appropriate.	partially fulfilled
	Administration of the test compound	
8	The duration of exposure was suitable for the test system and investigated endpoints.	fulfilled
9	The concentrations used were suitable for the test system and investigated endpoints.	fulfilled
10	The test conditions during and after exposure to the test compound were suitable (media and serum used, cell density, incubation temperature, humidity, CO2 concentration).	partially fulfilled
	Data collection and analysis	
11	Reliable and sensitive tests and/or analytical methods were used for investigating the endpoints.	fulfilled
12	Sufficient numbers of replicates or repetitions of the experiment were used to generate reliable and valid results.	fulfilled
13	Measurements were collected at suitable time points in order to generate sensitive, valid and reliable data.	fulfilled
14	Cytotoxicity was measured and the test compound did not cause cytotoxicity that significantly affected the results.	fulfilled
15	The statistical methods were clearly described and do not seem inappropriate, unusual or unfamiliar.	partially fulfilled
	Other	
16	Are there any other aspects of study design, performance or reporting that influence reliability?	

	RELEVANCE	**SELECTION**	**COMMENT**
1	The identity of the tested substance	directly relevant	
2	The test system used	directly relevant	
3	The endpoint studied	directly relevant	
4	The concentrations used	directly relevant	

	% FULFILLED CRITERIA	
	REPORTING	**METHOD**
Study overall	77.27	70.00
Test compounds and controls	60.00	40.00
Test system	58.33	75.00
Administration of test compound	100.00	83.33
Data collection and analysis	90.00	90.00
Funding and competing interests	100.00	





Article 20

Antimalarial activity of Garcinia mangostana L rind and its synergistic effect with artemisinin *in vitro*

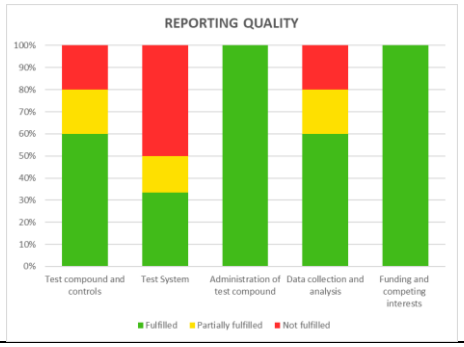
No.	REPORTING QUALITY	SELECTION	COMMENT
	Test compound and controls		
1	The chemical name or other identification, such as CAS-number, of the test compound was given.	fulfilled	
2	The purity of the test compound was stated or is traceable according to information given regarding manufacturer and lot/batch number. In case of mixtures, the composition of different constituents was stated.	partially fulfilled	
3	The solubility of the test compound was described.	fulfilled	
4	The solvent (vehicle) was described.	fulfilled	
5	It was stated that a solvent (vehicle) control was included.	not fulfilled	
	Test System		
6	The test system (e.g., cell line / cells/ tissue / organ / embryo / sub-cellular fractions) was described.	fulfilled	
7	The source of the test system was stated.	fulfilled	
8	The metabolic competence, i.e., competence of the test system to metabolize the test compound into an active metabolite was described.	not fulfilled	
9	The number of cell passages of the cell line used, was stated. (Remove this criterion if the study was not conducted in a cell line.)	REMOVE	
10	Composition of media was described, including use of serum, antibiotics, etc.	partially fulfilled	
11	Incubation temperature, humidity, and CO2 concentration were described.	not fulfilled	
12	Measures taken for avoiding or screening for contamination by mycoplasma, bacteria, fungi and virus were described.	not fulfilled	
	Administration of test compound		
13	The administered dose levels or concentrations were stated.	fulfilled	
14	Cell density or number of cells used during treatment was described. (Remove this criterion if the study was not conducted in a cell line.)	REMOVE	
15	The duration of treatment was stated.	fulfilled	
16	The number of replicates per dose level/concentration or the number of times the experiment was repeated was stated.	fulfilled	
	Data collection and analysis		
17	The tests and/or analytical methods used were sufficiently described to allow for evaluation of reliability of results.	fulfilled	
18	The time points for data collection were stated.	fulfilled	
19	It was stated that the effect of the test compound on cytotoxicity was measured.	not fulfilled	
20	All results were clearly presented.	fulfilled	
21	The statistical methods and software used were described.	partially fulfilled	
	Funding and competing interests		

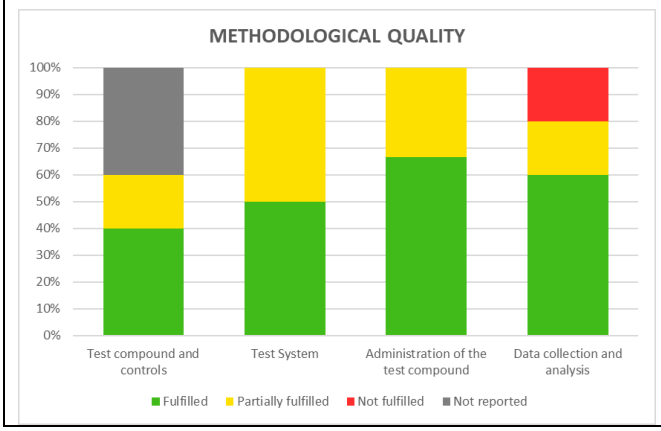
22	The funding sources for the study were stated.	fulfilled
23	Any competing interests were disclosed or it was explicitly stated that the authors did not have any competing interests.	fulfilled
	Other	
24	Was all information that is indispensable for evaluating the reliability of data given? This includes information on the test compound and controls, test system, study design or study performance.	

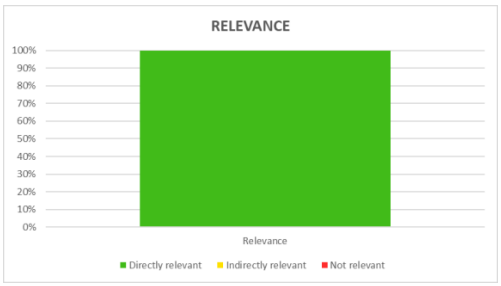
	METHODOLOGICAL QUALITY	SELECTION	COMMENT
	Test compound and controls		
1	The test compound or mixture was unlikely to contain any impurities that may significantly have affected the results of the study.	partially fulfilled	
2	It was likely that the test compound was soluble at the concentrations used.	fulfilled	
3	An appropriate solvent (vehicle) was used that is not expected to interfere with the results of the study at the concentration used.	fulfilled	
4	A solvent (vehicle) control was included.	not reported	
5	An appropriate positive control was included, and the expected result was observed from this treatment.	not reported	
	Test System		
6	A reliable and sensitive test system (e.g., cell line / cells/ tissue / organ / embryo / sub-cellular fractions) with metabolic competence, if relevant, was used for investigating the test compound and endpoints.	fulfilled	
7	Conditions for cultivation and/or maintenance of the cell line / cells / tissue / organ /embryo / sub-cellular fractions (incubation temperature, humidity, CO2 concentration, media used, number of cell passages, control of contamination) were appropriate.	partially fulfilled	
	Administration of the test compound		
8	The duration of exposure was suitable for the test system and investigated endpoints.	fulfilled	
9	The concentrations used were suitable for the test system and investigated endpoints.	fulfilled	
10	The test conditions during and after exposure to the test compound were suitable (media and serum used, cell density, incubation temperature, humidity, CO2 concentration).	partially fulfilled	
	Data collection and analysis		
11	Reliable and sensitive tests and/or analytical methods were used for investigating the endpoints.	fulfilled	
12	Sufficient numbers of replicates or repetitions of the experiment were used to generate reliable and valid results.	fulfilled	
13	Measurements were collected at suitable time points in order to generate sensitive, valid and reliable data.	fulfilled	
14	Cytotoxicity was measured and the test compound did not cause cytotoxicity that significantly affected the results.	not fulfilled	
15	The statistical methods were clearly described and do not seem inappropriate, unusual or unfamiliar.	partially fulfilled	
	Other		
16	Are there any other aspects of study design, performance or reporting that influence reliability?		

	RELEVANCE	SELECTION	COMMENT
1	The identity of the tested substance	directly relevant	
2	The test system used	directly relevant	
3	The endpoint studied	directly relevant	
4	The concentrations used	directly relevant	

	% FULFILLED CRITERIA	
	REPORTING	**METHOD**
Study overall	69.05	66.67
Test compounds and controls	70.00	50.00
Test system	41.67	75.00
Administration of test compound	100.00	83.33
Data collection and analysis	70.00	70.00
Funding and competing interests	100.00	







Annex 5. ToxRTool *in vivo* assay.

Rater

ToxRTool: A tool to assess the reliability of toxicological data		
Optional information for feed-back to ECVAM		
Date/period of evaluation:	May 19th, 2025	
Rater information		
Name	Joanne Theophilia Winata	
Address	Department of Pharmacy, Faculty Pharmacy, UGM, Sekip Utara, 55281, Yogyakarta, Indonesia	
Affiliation	UGM	
Education (subject and degree)	Pharmacy (Master)	
Experience in toxicology (years)	0	
Experience in evaluating toxicological studies and/or performing risk asessments (years)	0	
Please answer these questions **after** having peformed the evaluation!		
In general, do you consider a tool for assessing the reliability of toxicological data useful?	Yes	
Is the tool readily applicable and user-friendly?	Yes	
Is the tool transparent?	Yes	
How much time did you need (on average, or range) to read a case study and to apply the assessment tool to a study?	reading/understanding (minutes per study)	30
	tool application (minutes per study)	15
Did the application of the assessment tool influence/change your opinion on how to assess data quality? If yes, in which way?	Yes. The tool offered a structured framework that revealed overlooked aspects of data quality, like contextual relevance and methodological clarity.	
Did you find it helpful to have the possibility to document observations on relevance of data (fields below reliability criteria)?	Yes	
Do you have specific comments on individual criteria (it is not necessary to repeat here the comments you might have given in the case study worksheets)?	No	
Do you have other/general comments on the tool?	No	

Explanations

Explanations to ToxRTool
Objective: ToxRTool is designed to assess the inherent quality, also called reliability, of toxicological data as reported in a publication or a test report.
This tool essentially comprises a list of evaluation criteria. Criteria are subdivided in five groups:
I: Test substance identification, II: Test system characterisation, III: Study design description, IV: Study results documentation, V: Plausibility of study design and data
Per criterion either one ('1') or no ('0') point can be assigned. If a criterion is met, assign '1', if not assign '0'. Please choose from the respective drop-down list. **All criteria must be answered!**
In total 21 points for *in vivo* studies, 18 points for *in vitro* studies can be assigned. A reliability categorisation based on the total number of points is given below.
Criteria written in red have special importance: points for each of the red criteria are necessary to achieve Reliability category 1 or 2. **Please evaluate with special care!**
Data entry is requested in (and is also restricted to) the fields shaded in green.

Reliability categorisation (definition of categories according to Klimisch et al. 1997)				**(Proposed) consequence**
	in vivo	***in vitro***		
1	18-21	15-18	reliable without restrictions	useful, check relevance for intended purpose

2	13-17	11-14	reliable with restrictions	potentially useful, check relevance for intended purpose
3	<13 or not all **red** criteria met	<11 or not all **red** criteria met	not reliable	generally not to be used as key study, but depending on the shortcomings of the study it may still be useful in weight-of-evidence approaches or as supportive information
4			not assignable: documentation insufficient (reviews, handbooks, other secondary sources)	generally not to be used as key study, but depending on the shortcomings of the study it may still be useful in weight-of-evidence approaches or as supportive information. (This category is not an outcome of this evaluation tool!)
	In addition to the criteria for assessing data reliability, at the bottom of the worksheets there are some questions to be answered optionally ("Optional documentation of observations with importance to relevance"). These questions allow to document observations in a non-formalised way, which may be of importance for the further use of the information for regulatory or other purposes.			

Article 6

Reliability assessment of *in vivo* toxicity studies			
Study under evaluation			
Authors:			
	Joanne Theophilia Winata		
Title:			
	apt.		
Testing facility, year, sponsor, study no. or bibliographic reference:			
	Systematic review 2025		
Explanations are available for most criteria and show up, when the cursor is moved over the criteria field. Please read carefully! Red criteria: the maximum score is needed for these criteria to achieve reliability category 1 or 2 (see worksheet Explanations): Please evaluate with special care!			
Criteria			**Evaluator's explanations, comments on criteria, etc.**
No.	**Criteria Group I: Test substance identification**	**Score**	
1	Was the test substance identified?	1	
2	Is the purity of the substance given?	1	
3	Is information on the source/origin of the substance given?	1	
4	Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data (see explanation for examples)?	1	
		4	
	Criteria Group II: Test organism characterisation		
5	Is the species given?	1	
6	Is the sex of the test organism given?	0	
7	Is information given on the strain of test animals plus, if considered necessary to judge the study, other specifications (see explanation for examples)?	1	
8	Is age or body weight of the test organisms at the start of the study given?	1	
9	For repeated dose toxicity studies only (give point for other study types): Is information given on the housing or feeding conditions?	1	
		4	
	Criteria Group III: Study design description		
10	Is the administration route given?	1	
11	Are doses administered or concentrations in application media given?	1	
12	Are frequency and duration of exposure as well as time-points of observations explained?	1	
13	Were negative (where required) and positive controls (where required) included (give point also, when absent but not required, see explanations for study types and their respective requirements on controls)?	1	
14	Is the number of animals (in case of experimental human studies: number of test persons) per group given?	1	
15	Are sufficient details of the administration scheme given to judge the study (see explanation for examples)?	1	

16	For inhalation studies and repeated dose toxicity studies only (give point for other study types): Were achieved concentrations analytically verified or was stability of the test substance otherwise ensured or made plausible?	1	
		7	
	Criteria Group IV: Study results documentation		
17	Are the study endpoint(s) and their method(s) of determination clearly described?	1	
18	Is the description of the study results for all endpoints investigated transparent and complete?	1	
19	Are the statistical methods applied for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable, see explanations)?	1	
		3	
	Criteria Group V: Plausibility of study design and results		
20	Is the study design chosen appropriate for obtaining the substance-specific data aimed at (see explanations for details)?	1	
21	Are the quantitative study results reliable (see explanations for arguments)?	1	
		2	
		20	
	A Numerical result leads to initial Category:	**1**	
	B Checking red scores leads to revised Category:	**1**	
	C Evaluator's proposal: Category:	**2**	
	D Justification in case evaluator deviates from B:		
Optional documentation of observations with importance to relevance (not part of the reliability assessment)			
	During the course of the quality assessment observations may be made which are important for discussing the **relevance** of the data for specific purposes. The optional possibility is provided here to document these observations for future use.		
	What is the purpose of this quality evaluation (data documentation for use under REACH, classification activity under GHS, ECVAM validation activities, other)?		
	Study conducted according to recent OECD or EU guidelines (or other, e.g. national guidelines)? If yes, which ones? Study conducted under GLP conditions?		
	(If not a guideline study): Does a guideline exist for the study endpoint(s) under investigation?		
	Are you aware of relevant deviations from the guideline(s) in the study evaluated? If yes, which one?		
	Did you make observations with importance to the regulatory use of the data (example 1: evaluator may hint that a whole body inhalation study was performed with a substance, for which profound percutaneous absorption is expected or known, leading to substantial percutaneous uptake in addition to inhalation uptake; example 2: an Ames reversion assay was performed with strains able to identify frameshift mutations only or without external metabolic activation; example 3: evaluator is in possession of positive evidence that the results obtained with the *in vitro* study under evaluation, in conjunction with known toxicokinetic data, are useful to assess the nephrotoxicity of the substance in humans)?		
	Would you like to make other/general comments on the usability of the data?		

Article 11

Reliability assessment of *in vivo* toxicity studies	
Study under evaluation	
Authors:	
Joanne Theophilia Winata	
Title:	
apt.	
Testing facility, year, sponsor, study no. or bibliographic reference:	
Systematic review 2025	

Explanations are available for most criteria and show up, when the cursor is moved over the criteria field. Please read carefully! Red criteria: the maximum score is needed for these criteria to achieve reliability category 1 or 2 (see worksheet Explanations): Please evaluate with special care!			
Criteria			**Evaluator's explanations, comments on criteria, etc.**
No.	**Criteria Group I: Test substance identification**	**Score**	
1	Was the test substance identified?	1	
2	Is the purity of the substance given?	0	
3	Is information on the source/origin of the substance given?	1	
4	Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data (see explanation for examples)?	0	
		2	
	Criteria Group II: Test organism characterisation		
5	Is the species given?	1	
6	Is the sex of the test organism given?	0	
7	Is information given on the strain of test animals plus, if considered necessary to judge the study, other specifications (see explanation for examples)?	1	
8	Is age or body weight of the test organisms at the start of the study given?	1	
9	For repeated dose toxicity studies only (give point for other study types): Is information given on the housing or feeding conditions?	1	
		4	
	Criteria Group III: Study design description		
10	Is the administration route given?	1	
11	Are doses administered or concentrations in application media given?	1	
12	Are frequency and duration of exposure as well as time-points of observations explained?	1	
13	Were negative (where required) and positive controls (where required) included (give point also, when absent but not required, see explanations for study types and their respective requirements on controls)?	1	
14	Is the number of animals (in case of experimental human studies: number of test persons) per group given?	1	
15	Are sufficient details of the administration scheme given to judge the study (see explanation for examples)?	1	
16	For inhalation studies and repeated dose toxicity studies only (give point for other study types): Were achieved concentrations analytically verified or was stability of the test substance otherwise ensured or made plausible?	1	
		7	
	Criteria Group IV: Study results documentation		
17	Are the study endpoint(s) and their method(s) of determination clearly described?	1	
18	Is the description of the study results for all endpoints investigated transparent and complete?	1	
19	Are the statistical methods applied for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable, see explanations)?	1	
		3	
	Criteria Group V: Plausibility of study design and results		
20	Is the study design chosen appropriate for obtaining the substance-specific data aimed at (see explanations for details)?	1	
21	Are the quantitative study results reliable (see explanations for arguments)?	1	
		2	
		18	
	A Numerical result leads to initial Category:	**1**	
	B Checking red scores leads to revised Category:	**1**	
	C Evaluator's proposal: Category:	**3**	
	D Justification in case evaluator deviates from B:		
Optional documentation of observations with importance to relevance (not part of the reliability assessment)			
	During the course of the quality assessment observations may be made which are important for discussing the **relevance** of the data for specific purposes. The optional possibility is provided here to document these observations for future use.		

	What is the purpose of this quality evaluation (data documentation for use under REACH, classification activity under GHS, ECVAM validation activities, other)?		
	Study conducted according to recent OECD or EU guidelines (or other, e.g. national guidelines)? If yes, which ones? Study conducted under GLP conditions?		
	(If not a guideline study): Does a guideline exist for the study endpoint(s) under investigation?		
	Are you aware of relevant deviations from the guideline(s) in the study evaluated? If yes, which one?		
	Did you make observations with importance to the regulatory use of the data (example 1: evaluator may hint that a whole body inhalation study was performed with a substance, for which profound percutaneous absorption is expected or known, leading to substantial percutaneous uptake in addition to inhalation uptake; example 2: an Ames reversion assay was performed with strains able to identify frame-shift mutations only or without external metabolic activation; example 3: evaluator is in possession of positive evidence that the results obtained with the *in vitro* study under evaluation, in conjunction with known toxicokinetic data, are useful to assess the nephrotoxicity of the substance in humans)?		
	Would you like to make other/general comments on the usability of the data?		

Article 18

Reliability assessment of *in vivo* toxicity studies			
Study under evaluation			
Authors:			
	Joanne Theophilia Winata		
Title:			
	apt.		
Testing facility, year, sponsor, study no. or bibliographic reference:			
	Systematic review 2025		
Explanations are available for most criteria and show up, when the cursor is moved over the criteria field. Please read carefully! Red criteria: the maximum score is needed for these criteria to achieve reliability category 1 or 2 (see worksheet Explanations): Please evaluate with special care!			
Criteria			**Evaluator's explanations, comments on criteria, etc.**
No.	**Criteria Group I: Test substance identification**	**Score**	
1	Was the test substance identified?	1	
2	Is the purity of the substance given?	0	
3	Is information on the source/origin of the substance given?	1	
4	Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data (see explanation for examples)?	1	
		3	
	Criteria Group II: Test organism characterisation		
5	Is the species given?	1	
6	Is the sex of the test organism given?	1	
7	Is information given on the strain of test animals plus, if considered necessary to judge the study, other specifications (see explanation for examples)?	1	
8	Is age or body weight of the test organisms at the start of the study given?	1	
9	For repeated dose toxicity studies only (give point for other study types): Is information given on the housing or feeding conditions?	1	
		5	

	Criteria Group III: Study design description		
10	Is the administration route given?	1	
11	Are doses administered or concentrations in application media given?	1	
12	Are frequency and duration of exposure as well as time-points of observations explained?	1	
13	Were negative (where required) and positive controls (where required) included (give point also, when absent but not required, see explanations for study types and their respective requirements on controls)?	1	
14	Is the number of animals (in case of experimental human studies: number of test persons) per group given?	1	
15	Are sufficient details of the administration scheme given to judge the study (see explanation for examples)?	1	
16	For inhalation studies and repeated dose toxicity studies only (give point for other study types): Were achieved concentrations analytically verified or was stability of the test substance otherwise ensured or made plausible?	1	
		7	
	Criteria Group IV: Study results documentation		
17	Are the study endpoint(s) and their method(s) of determination clearly described?	1	
18	Is the description of the study results for all endpoints investigated transparent and complete?	1	
19	Are the statistical methods applied for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable, see explanations)?	1	
		3	
	Criteria Group V: Plausibility of study design and results		
20	Is the study design chosen appropriate for obtaining the substance-specific data aimed at (see explanations for details)?	1	
21	Are the quantitative study results reliable (see explanations for arguments)?	1	
		2	
		20	
	A Numerical result leads to initial Category:	**1**	
	B Checking red scores leads to revised Category:	**1**	
	C Evaluator's proposal: Category:	**3**	
	D Justification in case evaluator deviates from B:		
Optional documentation of observations with importance to relevance (not part of the reliability assessment)			
	During the course of the quality assessment observations may be made which are important for discussing the **relevance** of the data for specific purposes. The optional possibility is provided here to document these observations for future use.		
	What is the purpose of this quality evaluation (data documentation for use under REACH, classification activity under GHS, ECVAM validation activities, other)?		
	Study conducted according to recent OECD or EU guidelines (or other, e.g. national guidelines)? If yes, which ones? Study conducted under GLP conditions?		
	(If not a guideline study): Does a guideline exist for the study endpoint(s) under investigation?		
	Are you aware of relevant deviations from the guideline(s) in the study evaluated? If yes, which one?		

	Did you make observations with importance to the regulatory use of the data (example 1: evaluator may hint that a whole body inhalation study was performed with a substance, for which profound percutaneous absorption is expected or known, leading to substantial percutaneous uptake in addition to inhalation uptake; example 2: an Ames reversion assay was performed with strains able to identify frame-shift mutations only or without external metabolic activation; example 3: evaluator is in possession of positive evidence that the results obtained with the *in vitro* study under evaluation, in conjunction with known toxicokinetic data, are useful to assess the nephrotoxicity of the substance in humans)?		
	Would you like to make other/general comments on the usability of the data?		

Article 20

Reliability assessment of *in vivo* toxicity studies		
Study under evaluation		
Authors:		
Joanne Theophilia Winata		
Titel:		
apt.		
Testing facility, year, sponsor, study no. or bibliographic reference:		
Systematic review 2025		
Explanations are available for most criteria and show up, when the cursor is moved over the criteria field. Please read carefully! Red criteria: the maximum score is needed for these criteria to achieve reliability category 1 or 2 (see worksheet Explanations): Please evaluate with special care!		
Criteria		**Evaluator's explanations, comments on criteria, etc.**

No.	**Criteria Group I: Test substance identification**	**Score**	
1	Was the test substance identified?	1	
2	Is the purity of the substance given?	0	
3	Is information on the source/origin of the substance given?	1	
4	Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data (see explanation for examples)?	0	
		2	
	Criteria Group II: Test organism characterisation		
5	Is the species given?	1	
6	Is the sex of the test organism given?	1	
7	Is information given on the strain of test animals plus, if considered necessary to judge the study, other specifications (see explanation for examples)?	1	
8	Is age or body weight of the test organisms at the start of the study given?	1	
9	For repeated dose toxicity studies only (give point for other study types): Is information given on the housing or feeding conditions?	1	
		5	
	Criteria Group III: Study design description		
10	Is the administration route given?	1	
11	Are doses administered or concentrations in application media given?	1	
12	Are frequency and duration of exposure as well as time-points of observations explained?	1	
13	Were negative (where required) and positive controls (where required) included (give point also, when absent but not required, see explanations for study types and their respective requirements on controls)?	1	
14	Is the number of animals (in case of experimental human studies: number of test persons) per group given?	1	
15	Are sufficient details of the administration scheme given to judge the study (see explanation for examples)?	1	

16	For inhalation studies and repeated dose toxicity studies only (give point for other study types): Were achieved concentrations analytically verified or was stability of the test substance otherwise ensured or made plausible?	1	
		7	
	Criteria Group IV: Study results documentation		
17	Are the study endpoint(s) and their method(s) of determination clearly described?	1	
18	Is the description of the study results for all endpoints investigated transparent and complete?	1	
19	Are the statistical methods applied for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable, see explanations)?	1	
		3	
	Criteria Group V: Plausibility of study design and results		
20	Is the study design chosen appropriate for obtaining the substance-specific data aimed at (see explanations for details)?	1	
21	Are the quantitative study results reliable (see explanations for arguments)?	1	
		2	
		19	
	A Numerical result leads to initial Category:	**1**	
	B Checking red scores leads to revised Category:	**1**	
	C Evaluator's proposal: Category:	**2**	
	D Justification in case evaluator deviates from B:		
Optional documentation of observations with importance to relevance (not part of the reliability assessment)			
	During the course of the quality assessment observations may be made which are important for discussing the **relevance** of the data for specific purposes. The optional possibility is provided here to document these observations for future use.		
	What is the purpose of this quality evaluation (data documentation for use under REACH, classification activity under GHS, ECVAM validation activities, other)?		
	Study conducted according to recent OECD or EU guidelines (or other, e.g. national guidelines)? If yes, which ones? Study conducted under GLP conditions?		
	(If not a guideline study): Does a guideline exist for the study endpoint(s) under investigation?		
	Are you aware of relevant deviations from the guideline(s) in the study evaluated? If yes, which one?		
	Did you make observations with importance to the regulatory use of the data (example 1: evaluator may hint that a whole body inhalation study was performed with a substance, for which profound percutaneous absorption is expected or known, leading to substantial percutaneous uptake in addition to inhalation uptake; example 2: an Ames reversion assay was performed with strains able to identify frame-shift mutations only or without external metabolic activation; example 3: evaluator is in possession of positive evidence that the results obtained with the *in vitro* study under evaluation, in conjunction with known toxicokinetic data, are useful to assess the nephrotoxicity of the substance in humans)?		
	Would you like to make other/general comments on the usability of the data?		

Annex 6. Extraction template.

Article code	Title	Article citation	Country	Study design	Assay name	Compounds/ extracts/ fractions	Source	Plant	Subject/target assay	Test activity (IC_{50})	Comparators or positive controls	Control activity (IC_{50})	Cytotoxicity assay	Cytotoxicity (CC_{50}) of sample	SI	Note