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Review Article

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A decade of β-Hematin inhibition by plant extracts and polyphenol isolates: A narrative review of in vitro studies (2014–2024)

Abstract

Malaria remains one of the most persistent infectious diseases worldwide, and the spread of parasite resistance to chloroquine, sulfadoxine-pyrimethamine, and even artemisinin derivatives has renewed interest in plant-derived antimalarial leads. A widely used in vitro strategy for screening such leads targets the hemozoin-formation pathway, in which free ferriprotoporphyrin IX (heme), liberated during hemoglobin digestion by the intra-erythrocytic parasite, is bio-mineralized into the inert crystalline pigment hemozoin. Its synthetic analogue, β-hematin, is used as a convenient surrogate target in semi-quantitative colorimetric assays. This review compares ten in vitro studies (2014–2024) from our research group that examined the β-hematin-inhibitory activity of crude water and ethanolic extracts, preparative HPLC fractions, and pure phytochemical isolates obtained from *Artemisia annua*, *Inula viscosa*, grape (*Vitis vinifera*) peels and seeds, pomegranate (*Punica granatum*), turmeric (*Curcuma longa*), dietary flavonoid standards (morin, quercetin, catechin, quercitrin), guava (*Psidium guajava*), tea (*Camellia sinensis*), wild sage (*Salvia officinalis*) and dwarf nettle (*Urtica urens*). Across these studies, the extracts and isolates were evaluated using the Deharo semi-quantitative β-hematin inhibition assay, complemented by HPLC-PDA and UPLC/HPLC-ESI-MS profiling that identified flavonols, flavones, flavan-3-ols, curcuminoids, phenolic acids, ellagitannins and diterpenes as the principal marker constituents. Pomegranate leaves, black grape peels, wild-sage bicarbonate extracts and *Inula viscosa* crude extract emerged as the most potent preparations, rivalling or exceeding chloroquine at pharmacologically relevant concentrations, while Cameroon-sourced *Artemisia annua*, curcumin and dwarf nettle showed intermediate-to-modest activity and several matrices (white grape, pomegranate juice, green tea prepared in water, and nettle roots/stems) were essentially inactive. Sodium bicarbonate pretreatment consistently enhanced and stabilized activity, most strikingly converting an inactive green-tea water infusion into one comparable to chloroquine. Mechanistically, the reviewed data support a model in which polyphenolic hydroxyl groups engage the propionate side chains of free heme through hydrogen bonding, while aromatic rings stack against the porphyrin macrocycle, together blocking the hemozoin crystal growth face and precipitating oxidative parasite death. The consistent superiority of crude or semi-purified extracts over several single isolates further points to synergistic phytochemical interactions. Because β-hematin inhibition is a preliminary biochemical screen and not a validated surrogate for antiplasmodial efficacy, the rankings and mechanistic conclusions offered here should be read as hypothesis-generating rather than confirmatory.

Keywords: antimalarial activity, β-hematin inhibition, hemozoin, flavonoids, polyphenols, crude plant extracts; HPLC-PDA, LC-MS, sodium bicarbonate, natural products

Volume 14 Issue 4 - 2026

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Received: July 28, 2026 | **Published:** September 11, 2026

Abbreviations: ACTs, artemisinin-based combination therapies; UPLC, ultra performance liquid chromatography; HPLC, high performance liquid chromatography; LC-MS, liquid chromatography-mass spectrometry; PDA, photodiode array; ESI, electrospray ionization; MS, mass spectrometry; QDa, quadrupole mass analyzer detector; TSQ, triple-stage quadrupole; ODS, octadecylsilane; EGCG, epigallocatechin-3-gallate; EGC, epigallocatechin; ECG, epicatechin-3-gallate; EC, epicatechin; DMSO, dimethyl sulfoxide; $NaHCO_3$, sodium bicarbonate; CQ, chloroquine diphosphate salt; ACN, acetonitrile; FA, formic acid; H_2O, water; NaOH, sodium hydroxide; UV, ultraviolet; UV-Vis, ultraviolet-visible spectroscopy; ELISA, enzyme-linked immunosorbent assay; HDP, heme detoxification protein; HHDP, hexahydroxydiphenoyl; M, molar; mL, milliliter; mg, milligram; mg/mL, milligrams per milliliter; mM, millimolar; nm, nanometer; °C, degrees Celsius; A405, absorbance at 405 nm; $[M-H]^-$, deprotonated molecular ion; $[M+H]^+$, protonated molecular ion

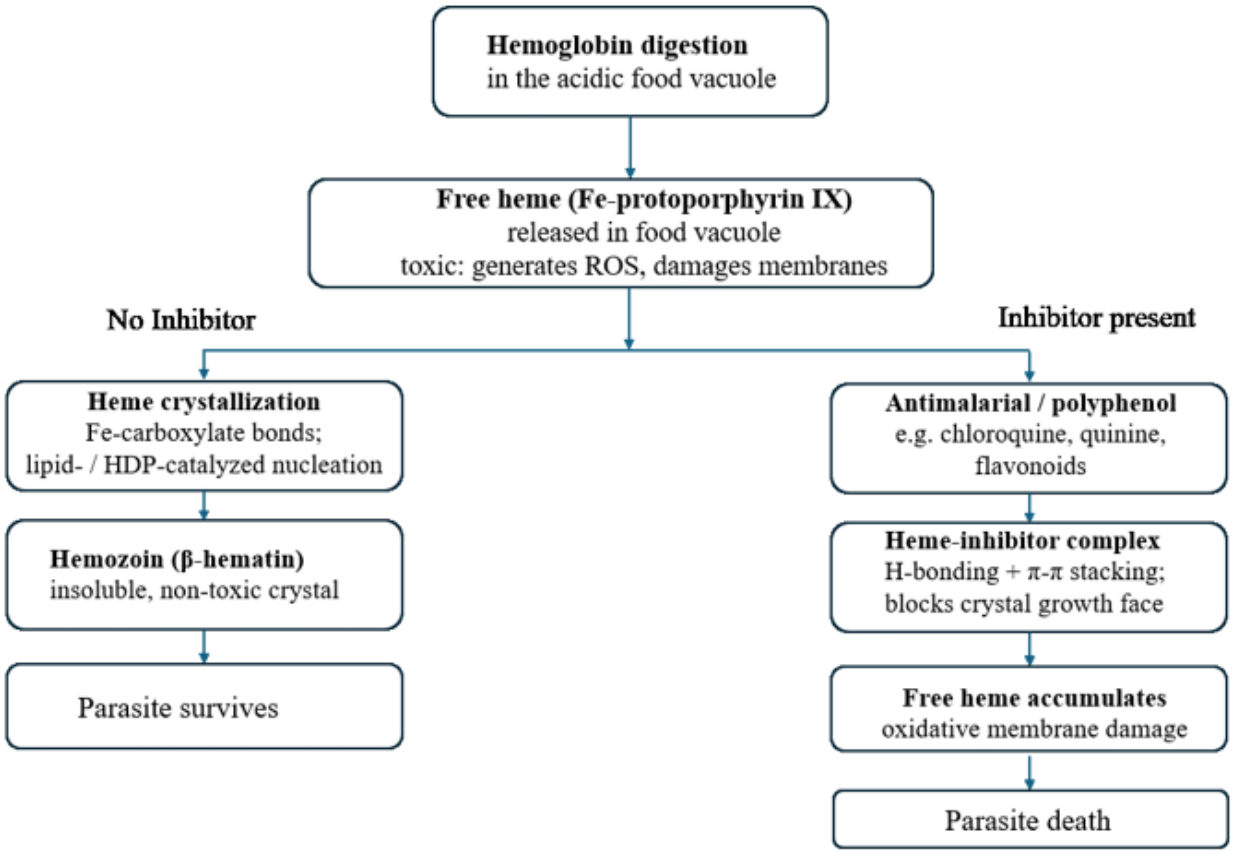
Introduction

Malaria continues to impose an enormous global health burden. According to the World Malaria Report 2025, an estimated 282 million cases and 610,000 malaria-related deaths occurred in 2024, an increase of roughly 9 million cases compared with 2023; the WHO African Region accounts for approximately 94% of cases and 95% of deaths, with children under five years bearing about 75% of the regional mortality.[1] Progress achieved over the previous two decades through insecticide-treated nets, indoor residual spraying and improved diagnostics has stalled in recent years because of insecticide and drug resistance, climate change, humanitarian crises and funding shortfalls.[1] The disease is caused by protozoan parasites of the genus Plasmodium transmitted by infected female Anopheles mosquitoes; of the five species infecting humans, *Plasmodium falciparum* is the most virulent and responsible for most of the severe disease and death.[1,2]

The emergence and spread of parasite resistance to nearly every major antimalarial class, chloroquine, sulfadoxine-pyrimethamine, and the artemisinin-based combination therapies (ACTs), has created an urgent need for antimalarial agents with new chemical scaffolds and mechanisms of action. Natural products have historically been the single most productive source of such agents: quinine, isolated from Cinchona bark, and artemisinin, isolated from the Chinese herb Artemisia annua, both transformed malaria chemotherapy and remain the structural ancestors of drugs in clinical use today.[2] This history is a large part of why plant extracts and foods, and the flavonoids, phenolic acids, tannins, curcuminoids and terpenoids isolated from them, are still routinely screened for antimalarial leads that could be developed further; any interim therapeutic use would first need the kind of confirmatory efficacy and safety data discussed later in the challenges, limitations and future perspectives section.

A mechanistically well-defined and experimentally tractable target for such screening is the hemozoin formation pathway. During the intra-erythrocytic (trophozoite) stage of its life cycle, Plasmodium digests host hemoglobin within an acidic food vacuole using a battery of proteases (plasmepsins, falcipains, and a histo-aspartic protease), liberating large quantities of free ferriprotoporphyrin IX (heme).[3] Free heme is toxic to the parasite because its redox-active iron center catalyzes the generation of reactive oxygen species and damages membranes and enzymes. To survive this self-generated toxic load, the parasite converts heme into an inert, insoluble crystalline polymer known as hemozoin ('malaria pigment'), in which adjacent ferric-heme units are linked through reciprocal iron–carboxylate bonds between the central iron of one heme and a propionate oxygen of its neighbor; crystallization is thought to be nucleated at lipid interfaces and/or facilitated by a parasite heme detoxification protein (HDP).[3] Because this detoxification step is essential for parasite survival, chloroquine and structurally related quinoline drugs are believed to act chiefly by binding free heme and blocking the growing hemozoin crystal face, causing toxic heme to accumulate and killing the parasite.[3,4] A synthetic analogue of hemozoin, β-hematin, is spectroscopically, crystallographically and chemically identical to the native purified pigment and can be produced in vitro under mildly acidic conditions; consequently, inhibition of β-hematin formation is now a standard surrogate assay for antimalarial screening, which was used across every study considered in this review.

The proposed mode of action of polyphenolic and other natural inhibitors within this pathway is summarized in Scheme 1. In the absence of an inhibitor, heme released during hemoglobin digestion is efficiently bio-crystallized into hemozoin and the parasite survives. When a polyphenol or quinoline-type inhibitor is present, it forms a heme–inhibitor complex stabilized principally by hydrogen bonds between the compound's hydroxyl/carbonyl groups and the free propionate side chains of heme, reinforced by hydrophobic π–π stacking between aromatic/porphyrin rings.[3–14] This complex caps the hemozoin crystal growth face and prevents further heme incorporation, so that unpolymerized free heme accumulates, generates oxidative membrane damage, and ultimately kills the parasite. This shared mechanistic rationale underlies the consistent use of the β-hematin inhibition assay as a first-tier screen in all ten primary studies reviewed here.



Scheme 1 Mechanism of heme detoxification and inhibition in plasmodium

Citation: Abu-Lafi S, Akkawi Z, Akkawi M. A decade of β-Hematin inhibition by plant extracts and polyphenol isolates: A narrative review of in vitro studies (2014–2024). *Pharm Pharmacol Int J.* 2026;14(4):117–125. DOI: 10.15406/ppij.2026.14.00503

The ten primary studies included in this review[5–14] span a decade of work (2014–2024) from a coordinated research program and provide an unusually coherent dataset for cross-comparison, since nearly all employed the same Deharo assay,[15] similar chloroquine (and, in several cases, amodiaquine) positive controls, and complementary HPLC-PDA or UPLC/HPLC–ESI-MS chromatographic identification of active constituents. The principal marker compounds reported across these ten sources are summarized schematically in Figure 1.

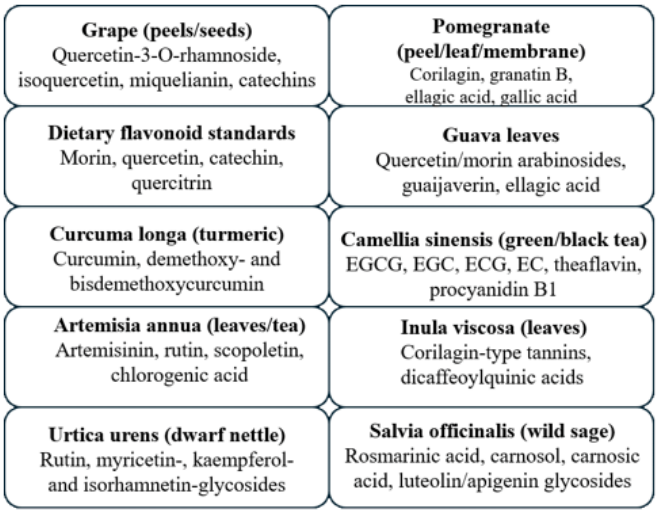


Figure 1 Overview of the ten plant/food matrices reviewed, and their representative marker phytochemicals identified by HPLC-PDA-ESI-MS across the ten primary studies. Full compound listings and structural classification are provided in Table 3.

This review sets out to do three things: bring together and compare the in vitro β-hematin-inhibitory activity reported for crude extracts, HPLC fractions and pure isolates across ten plant and food sources studied under a common experimental framework; rank these sources by relative antimalarial potency and classify the phytochemicals behind that activity; and evaluate the methodological strengths and weaknesses of this body of work – including the notable effect of sodium bicarbonate on extraction efficiency and stability – so we can point to concrete next steps for turning these leads into validated antimalarial candidates.

Methodology and study design

This review is based on a narrative synthesis of ten primary research articles,[5–14] all originating from the same collaborative research group and published in peer-reviewed pharmacy/pharmacology journals between 2014 and 2024. Studies were included because they (a) tested a plant-, food- or standard-derived extract, HPLC fraction or pure phytochemical for its ability to inhibit β-hematin formation in vitro, and (b) reported the chemical identity of at least the major constituents by chromatographic and/or mass-spectrometric means; no studies meeting these criteria were excluded. No new experimental work was performed; rather, absorbance values, dilution/concentration series and compound identifications reported in the original papers were extracted, tabulated and cross-compared.

Scope and study identification: This review is a narrative review, not a systematic one in the PRISMA sense. We deliberately limited it to our own group's ten β-hematin studies from Al-Quds University (2014–2024), because, as far as we can tell, no other laboratory – in Palestine or anywhere else – has run the same assay across this many plant, food and phytochemical matrices, and it is exactly that shared protocol that makes a direct comparison possible. To make sure we had not missed anything, we also searched PubMed, Google Scholar and Scopus (up to 2025) for "β-hematin" or "hemozoin inhibition" combined with "plant extract, and "polyphenol".

Study design across the ten papers followed a remarkably consistent template, summarized below.

Extraction: Plant material (leaves, peels, seeds, roots, stems, membranes or whole fruit/juice, depending on the source) was air- or shade-dried, powdered, and extracted either as a hot-water infusion (2 g in 150 mL distilled water at 90 °C for 15–20 min, mimicking traditional herbal-tea preparation), as an ethanolic or hydro-ethanolic maceration (typically 35% or absolute ethanol), or, for wild sage and green tea, as a sodium bicarbonate ($NaHCO_3$) infusion designed to test the effect of pH/alkalinity on extraction efficiency. Extracts were concentrated under reduced pressure and freeze-dried to constant weight before storage in amber/opaque containers.[5–14]

Semi-quantitative β-hematin inhibition assay: All ten studies used the Deharo et al. non-radiolabeled ferriprotoporphyrin IX biomineralization inhibition assay, or a minor modification of it.[15] Briefly, hemin chloride (freshly dissolved in DMSO, 0.5 mg/mL) was combined with 0.5 M sodium acetate buffer (pH 4.4), and the test extract/isolate or a control was added strictly in that order to a 96-well plate, then incubated at 37 °C for 18–24 h to allow acid-catalyzed β-hematin biomineralization. Plates were centrifuged, the supernatant discarded, and wells washed with DMSO to remove unreacted hemin; the remaining β-hematin was dissolved in 0.1 M NaOH to regenerate alkaline hematin, which was read spectrophotometrically at 405 nm on an ELISA reader. Ultrapure water served as the negative control, and chloroquine (typically 0.1 mg/mL) with amodiaquine as a secondary positive control in several studies. Because absorbance at 405 nm is inversely proportional to the amount of β-hematin inhibited, a lower reading denotes greater antimalarial efficiency; each reported value is the mean of 16 independent replicates.[5–14]

Quantitative assay: For selected pure isolates (curcumin, morin, quercetin), a complementary gravimetric method[16] determined the percentage yield of β-hematin formed in the presence of the test compound relative to DMSO and amodiaquine controls, from which an "efficiency" (%) was calculated; this cross-validated the semi-quantitative absorbance data.[8,9]

Chromatographic and mass-spectrometric profiling: Analytical HPLC-PDA (Waters Alliance e2695/2996, or Acquity UPLC H-Class systems) and preparative HPLC (3535 quaternary/Agilent PrepHT C18 columns) were used to separate and quantify major peaks, typically on reversed-phase ODS/C18 columns with acetic-acid–water/acetonitrile gradients and PDA detection between 200–500 nm. In the more recent studies (2019–2024), UPLC/HPLC coupled to an Acquity QDa or TSQ Quantum Access MAX mass spectrometer, operated in positive and negative electrospray ionisation (ESI) full-scan mode, allowed tentative identification of separated compounds from their $[M-H]^-$/$[M+H]^+$ pseudo-molecular ions and UV–Vis maxima, cross-referenced against published spectral libraries.[5–14]

Because the ten studies were not designed a priori as a single comparative program, several methodological variables differ between them and constrain direct cross-comparison: extract concentration is reported variously as a percentage dilution of a fixed stock, as mg/mL of dry extract, or pure compound; alkaline hematin absorbance values with chloroquine as a positive control, though always the principal benchmark.

Results and discussion

In vitro antimalarial activity of the reviewed plants, isolates and foods

Table 1 summarizes, for each of the ten sources, the family, plant part(s) and extraction mode used, the major identified active compounds, and the concentration/dilution range over which β-hematin inhibition remained comparable to (or better than) the chloroquine positive control.

Comparative analysis of the ten studies

Read together, the ten studies converge on several recurring findings. First, geographic origin and cultivar/chemotype strongly modulate activity within the same species: Cameroon-grown *Artemisia annua*, richer in artemisinin, rutin and scopoletin, inhibited β-hematin formation roughly twenty-fold more potently than the chlorogenic-acid-rich Luxembourg chemotype, and an analogous pattern distinguished the strongly active black/shami grape cultivars from the inactive white cultivar, whose peel, seed, juice and wine all failed to inhibit β-hematin, most likely reflecting the near-absence of anthocyanins and lower flavan-3-ol/flavonol content in white grapes.[6,12] Second, plant part matters as much as species: pomegranate leaves and peel were markedly more active than the membrane, and plain pomegranate juice - unlike the concentrated peel, leaf and membrane extracts - was essentially inactive;[7] likewise, nettle leaves retained useful activity down to 0.5 mg/mL while the roots and stems of the same plant showed minimal effect.[14]

Third, and perhaps most consistently, crude or minimally processed extracts frequently matched or outperformed their own purified fractions or isolated marker compounds. The clearest demonstration is *Inula viscosa*, whose whole 35% ethanolic extract (~93% inhibition) exceeded every one of its nine preparative HPLC fractions, including the three (IV, V, VI) that retained measurable activity, and outperformed the isolated pure compounds obtained from those fractions - a pattern attributed to synergistic interactions among co-occurring phenolics.[5] A directly comparable hierarchy - crude extract > fraction > isolate - was not universal, however: for turmeric, the isolated curcumin standard was substantially more potent than the crude ethanolic extract (roughly ten-fold), implying that in this matrix curcumin is diluted rather than potentiated by co-extracted material.[8] Pure dietary flavonoid standards (morin, quercetin, catechin, quercitrin) were, in isolation, highly active and in the case of morin rivalled chloroquine and amodiaquine down to 0.04 mg/mL,[9] underscoring that the crude-versus-isolate relationship is compound- and matrix-dependent rather than a fixed rule.

Fourth, several studies specifically demonstrated that a simple aqueous infusion - the mode of preparation most accessible to resource-limited populations who rely on herbal teas - can rival chloroquine, notably for pomegranate leaves/peel, black grape peel, black tea, and wild sage, reinforcing the practical relevance of these findings for affordable, candidate matrices for future community-level evaluation, pending confirmatory efficacy and safety testing.[7,6,11,13] Fifth, UPLC-MS profiling across the studies repeatedly identified members of the same core phytochemical families-quercetin-, kaempferol-, and morin-type flavonol glycosides; catechins and their gallate esters; hydroxycinnamic and ellagitannin-derived phenolic acids - as the recurring markers of activity (detailed in Table 3), lending internal consistency to the proposed heme-binding mechanism common to nearly every source examined.

Ranking of relative in vitro antimalarial potency

Because absolute absorbance values are not strictly comparable across independently run assays, the sources were ranked qualitatively using, for each study, the concentration/dilution range over which activity remained equal to or better than the chloroquine control measured in that same study (as tabulated using the normalized activity data underlying Table 1).

Table 1 In vitro antimalarial activity of the plants, isolates and foods reviewed, based on β-hematin (alkaline hematin) absorbance at 405 nm relative to the chloroquine (CQ) positive control reported in each primary study.[5–14] A405 values are inversely proportional to antimalarial efficiency.

Source	Family	Part / extract	Major active compounds	Activity vs. chloroquine (CQ)	Ref.
Artemisia annua (Cameroon vs. Luxembourg)	Asteraceae	Leaves, water infusion/tea	Artemisinin, rutin, scopoletin, chlorogenic acid; flavonoids/ polyphenols	Cameroon extract: A405 = 0.075 at 10% dilution (CQ = 0.058), i.e. comparable to CQ; Luxembourg extract: A405 = 1.515 at 10% (~20-fold less potent, essentially inactive)	12
Inula viscosa	Asteraceae	Leaves, 35% ethanolic extract; prep-HPLC fractions	Mono- and dicaffeoylquinic (chlorogenic-type) acids, sakuranetin, other flavonoids	Crude extract: ~93% β-hematin inhibition (CQ = 94%); only fractions IV,V,VI of 9 active; crude extract outperformed both fractions and pure isolates and remained stable for >3 years	5
Grape (black, shami, white)	Vitaceae	Peels and seeds, water extracts (5 mg/mL)	Quercetin-3-O-rhamnoside, isoquercitrin, miquelianin, syringetin glucoside, caffeic acid, catechins	Black grape peel (100%): A405 = 0.104, better than CQ (0.107); black/shami seed extracts remained active down to 5% dilution; white grape peel/seed, all juices and wines were inactive	6
Pomegranate (juice, peel, leaf, membrane)	Lythraceae	Juice, peel, leaf, membrane; water infusion	Corilagin, brevifolin carboxylic acid, galloyl-HHDP-hexoside, granatin B, ellagic acid, eschweilenol C	Leaf extract most potent, remaining ≤ CQ from 100% to 20% dilution (A405 0.055–0.073 vs. CQ 0.077); peel extract active to 30% dilution (0.078); plain (unconcentrated) juice was inactive	7

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Table 1 Continued..

Curcuma longa (turmeric)	Zingiberaceae	Rhizome, ethanolic extract; isolated curcuminoids	Curcumin, demethoxycurcumin, bisdemethoxycurcumin	Curcumin standard at 0.1 mg/mL: A405 = 0.146 (CQ = 0.107); efficiency 78.8% vs. 91.8% for amodiaquine (0.4 mM); crude ethanolic extract ~10-fold less active than CQ	8
Isolated dietary flavonoids (morin, quercetin, catechin, quercitrin)	(analytical standards)	Pure isolated compounds	Morin, quercetin, catechin, quercitrin hydrate	Morin most potent, active down to 0.04 mg/mL (A405 = 0.136, comparable to CQ 0.070 / amodiaquine 0.067); quercetin, catechin and quercitrin also active near 0.1 mg/mL (A405 ≈ 0.079–0.085); gallic acid inactive even at 1.0 mg/mL	9
Guava (Psidium guajava)	Myrtaceae	Leaves, water and 35% ethanolic extracts	Quercetin-3-O-glycoside, quercetin-galloylhexoside, morin-3-O-lyxoside/arabinoside, guaijaverin, ellagic acid	35% ethanolic extract active from 1 to 0.4 mg/mL (A405 0.065–0.082, vs. CQ 0.063); water extract active to 30% dilution (A405 ≈ 0.094–0.112)	10
Camellia sinensis (black and green tea)	Theaceae	Leaves, black and green tea water infusions (± $NaHCO_3$)	EGCG, EGC, ECG, EC, gallocatechin, theaflavin, procyanidin B1, gallic acid, quercetin-3-O-glycosides	Black tea 100% water extracts: A405 = 0.143–0.234 (CQ = 0.065), active even undiluted; green tea water infusion inactive (A405 ≈ 2.1) but became CQ-comparable (A405 = 0.062–0.082) down to 10% dilution when prepared with $NaHCO_3$	11
Wild sage (*Salvia officinalis*)	Lamiaceae	Leaves, water and $NaHCO_3$ extracts	Hispidulin-7-glucuronide, luteolin-7-O-rutinoside/ glucuronide, apigenin-7-O-glucoside, rosmarinic acid, carnosol, carnosic acid, isorhamnetin, hesperetin	$NaHCO_3$ extract (100%, day 1): A405 = 0.049, comparable to CQ (0.059); remained active to 30% dilution with $NaHCO_3$, versus rapid loss of activity for plain water extract on dilution; efficacy declined only marginally over 9 days storage	13
Dwarf/annual nettle (Urtica urens)	Urticaceae	Leaves (active); roots and stems (largely inactive); water extracts	Myricetin-3-O-rutinoside, isorhamnetin-, kaempferol- and quercetin- (rutin) glycosides, apigenin- and luteolin-diglucuronides	Leaf extract active from 1 to 0.5 mg/ mL (A405 = 0.088–0.156 vs. CQ 0.069) and down to 50% dilution; lost activity at 0.3 mg/mL and below 40% dilution; roots/stems showed minimal activity	14

On this basis, the ten sources rank, from most to least potent, approximately as follows:

1. Pomegranate leaf extract - active from 100% down to 20% dilution, matching or exceeding CQ across nearly the entire range and remaining well below the water blank even at 5% dilution.[7]
2. Black grape peel/seed extracts - exceed CQ at full strength and black-cultivar seed extracts remain active to 5% dilution; the white cultivar, by contrast, is completely inactive.[6]
3. Wild sage – $NaHCO_3$ extract - matches CQ at full strength and stays comparable down to 30% dilution, with only marginal loss of potency over nine days of storage.[13]
4. *Inula viscosa* crude ethanolic extract - ~93% β-hematin inhibition, essentially equal to the 94% achieved by CQ, and notably stable on storage (>3 years).[15]
5. Guava 35% ethanolic leaf extract - CQ-comparable between 1 and 0.4 mg/mL.[10]
6. Black tea water extract - potent undiluted, though 2–3.5-fold less active than CQ at equivalent concentration; $NaHCO_3$-processed green tea becomes fully CQ-comparable down to 10% dilution.[11]
7. Morin (isolated flavonol) - the most potent of the pure isolated flavonoids, remaining active to 0.04 mg/mL, essentially matching CQ/amodiaquine.[9]
8. *Artemisia annua*, Cameroon chemotype - comparable to CQ at 10% dilution; the Luxembourg chemotype of the same species is roughly twenty-fold weaker.[12]
9. Curcumin/turmeric - the pure curcumin standard is active at 0.1 mg/mL, but the crude ethanolic extract is about ten-fold less potent than CQ.[8]
10. Dwarf nettle (*Urtica urens*) leaf extract - the weakest of the ten, requiring comparatively high concentrations (1-0.5 mg/mL) and losing activity below 0.3 mg/mL or 40% dilution; root and stem extracts of the same plant were essentially inactive.[14]

This ranking should be read as indicative rather than absolute: it reflects concentration-normalized potency relative to chloroquine within each original study, not a single head-to-head experiment, and several "inactive" matrices within an otherwise active source (white grape, pomegranate juice, nettle root/stem, green tea in plain water) are excluded from the ranking of their respective species' best-performing preparation.

Effect of sodium bicarbonate ($NaHCO_3$) on antimalarial activity

Two of the ten studies directly compared plain hot-water infusions with infusions prepared using sodium bicarbonate as the extraction

medium: wild sage (*Salvia officinalis*) leaves[13] and green tea (*Camellia sinensis*, Twinings)[11] In both cases, the alkaline extraction consistently increased and stabilised β-hematin inhibitory activity relative to the corresponding plain water infusion, as summarized in Table 2.

Table 2 Effect of sodium bicarbonate ($NaHCO_3$) extraction on β-hematin inhibitory activity (absorbance at 405 nm) of wild sage and green tea, compared with plain water infusions and the chloroquine (CQ) positive control.[11,13]

Plant	Condition	Water extract A405 (405 nm)	$NaHCO_3$ extract A405 (405 nm)	CQ (0.1 mg/mL)	More active preparation
Wild sage (leaves)	Day 1, 100%	0.056	0.049	0.059	$NaHCO_3$ ≈ CQ
Wild sage (leaves)	Day 1, 50%	0.344	0.06	0.059	$NaHCO_3$ (water losing activity)
Wild sage (leaves)	Day 1, 30%	0.549	0.081	0.059	$NaHCO_3$ (water largely inactive)
Wild sage (leaves)	Day 9, 100%	0.09	0.08	0.062	Comparable, both ≈ CQ
Wild sage (leaves)	Day 9, 50%	1.19	0.071	0.062	$NaHCO_3$ (water inactive)
Wild sage (leaves)	Day 9, 30%	1.531	0.167	0.062	$NaHCO_3$ (water inactive)
Green tea	100%	2.110 (inactive)	0.062	0.069	$NaHCO_3$ only - comparable to CQ
Green tea	50%	not tested	0.075	0.069	$NaHCO_3$ ≈ CQ
Green tea	30%	not tested	0.076	0.069	$NaHCO_3$ ≈ CQ
Green tea	10%	not tested	0.082	0.069	$NaHCO_3$ ≈ CQ
Green tea	5%	not tested	1.198	0.069	Activity lost at this dilution
Green tea	0.10%	not tested	2.223	0.069	Inactive

Two patterns are evident. At full (100%) concentration, plain water infusions can be roughly as active as bicarbonate infusions for wild sage, but for green tea the plain water infusion was essentially inactive even undiluted (A405 ≈ 2.1, indistinguishable from the water blank).[11] The real advantage of bicarbonate emerges on dilution: $NaHCO_3$ extracts of both plants remained comparable to chloroquine down to 30% dilution (sage) or even 10% dilution (tea), whereas the corresponding water-only extracts lost most of their activity once diluted below 100% - for sage, activity fell 6- to 25-fold between 100% and 30-50% dilution. For sage, this advantage persisted over nine days of storage, with the $NaHCO_3$ extract declining only marginally while the water extract continued to weaken markedly.[13] The mechanistic interpretation offered in both papers is that the alkaline medium increases the solubility and extraction yield of polyphenolic actives and, for tea specifically, is believed to accelerate the autooxidation of catechins in a manner that favors heme complexation - effectively converting green tea catechins toward a black-tea-like, more strongly heme-reactive profile.[11] Practically, these results suggest that simple, low-cost modification of traditional herbal-tea preparation (adding a pinch of sodium bicarbonate, as is already customary in some regional tea-making practices) could enhance the antimalarial potential of an otherwise weak or inactive infusion, a low-technology laboratory finding that would need confirmation in live-parasite and toxicity assays before any community-level recommendation could be considered.

Classification of the identified phytochemicals

Table 3 groups the compounds tentatively or definitively identified by HPLC-PDA and UPLC-ESI-MS across the ten studies into structural classes. The classification highlights that flavonols and their O-glycosides (quercetin-, kaempferol-, morin- and myricetin-type) are by far the most widely and repeatedly detected family, followed by flavones (luteolin-, apigenin- and hispidulin-type), flavan-3-ols/catechins and their gallate esters, and a diverse set of phenolic acids, including hydroxycinnamic acids and ellagitannin-derived structures.

Table 3 Structural classification of the phytochemicals identified by HPLC-PDA/UPLC-ESI-MS across the ten reviewed studies.[5–14] Where a compound was named as an aglycone (e.g., quercetin, morin, kaempferol), it is grouped under the parent flavonol subclass together with its O-glycosides, since the 3-hydroxyflavone C-ring is common to the proposed heme-binding pharmacophore.

Phytochemical class	Representative compound(s)	Source (ref.)
Flavonols and O-glycosides	Quercetin	Grape;[6] morin/quercetin standards;[9] guava;[10] tea[11]
	Quercitrin (quercetin-3-O-rhamnoside)	Morin/quercetin standards[9]
	Quercetin-3-O-rhamnoside; -glucoside (isoquercitrin); -glucuronide (miquelianin); syringetin-3-(6-acetyl) glucoside	Grape[6]
	Quercetin-3-O-glycoside; quercetin-galloylhexoside; quercetin-3-O-arabinoside (guaijaverin)	Guava[10]
	Quercetin-3-O-galactosyl/glucosyl-rhamnosyl-glucoside; quercetin-3-O-rutinoside (rutin)	Tea;[11] nettle[14]
	Morin; morin-3-O-lyxoside; morin-3-O-arabinoside	Morin/quercetin standards;[9] guava[10]
	Kaempferol-3-O-β-D-xyloside	Pomegranate[7]
	Kaempferol-3-O-glucosyl-rhamnosyl-glucoside	Tea[11]
	Kaempferol 3,7-diglucoside; kaempferol-3-O-rutinoside; kaempferol 3-O-(6″-acetyl-galactoside) 7-O-rhamnoside	Nettle[14]
	Myricetin-3-O-rutinoside	Nettle[14]
	Isorhamnetin; isorhamnetin-3-O-glucoside-7-O-rhamnoside	Sage;[13] nettle[14]

Citation: Abu-Lafi S, Akkawi Z, Akkawi M. A decade of β-Hematin inhibition by plant extracts and polyphenol isolates: A narrative review of in vitro studies (2014–2024). *Pharm Pharmacol Int J.* 2026;14(4):117–125. DOI: 10.15406/ppij.2026.14.00503

Table 3 Continued...

Flavones and O-/C-glycosides	Apigenin-7-O-glucoside; apigenin-7-O-diglucuronide	Sage;[13] nettle[14]
	Luteolin-7-O-rutinoside; luteolin-7-O-glucuronide; luteolin-7-O-diglucuronide	Sage;[13] nettle[14]
	Hispidulin; hispidulin-7-glucuronide; pectolinarigenin; genkwanin	Sage[13]
	4′-O-Glucosylvitexin (apigenin C-glycoside)	Tea[11]
Flavan-3-ols, gallate esters and proanthocyanidins	Catechin	Morin/quercetin standards[9]
	Gallocatechin, epigallocatechin (EGC), epigallocatechin gallate (EGCG), epicatechin gallate (ECG)	Tea[11]
	Procyanidin B1 (catechin dimer)	Tea[11]
	Theaflavin (oxidative catechin dimer)	Tea[11]
Flavanones and flavanonols	Sakuranetin	Inula viscosa[5]
	Hesperetin	Sage[13]
	Astilbin (flavanonol glycoside, tentative)	Grape[6]
Anthocyanins	Cyanidin-3,5-diglucoside	Pomegranate[7]
Curcuminoids	Curcumin, demethoxycurcumin, bisdemethoxycurcumin	Turmeric[8]
Phenolic diterpenes (abietane-type)	Carnosol, carnosic acid, epirosmanol, rosmaridiphenol	Sage[13]
Lignans	Secoisolariciresinol hexoside	Pomegranate[7]
Xanthone glycosides	Eschweilenol C	Pomegranate[7]
Purine alkaloids	Theobromine	Tea[11]
Hydroxybenzoic acids	Gallic acid	Pomegranate;[7] morin/quercetin standards;[9] tea;[11] grape[6]
	p-Hydroxybenzoic acid (tentative)	Grape[6]
	Quinic acid; galloyl-hexoside; 5-O-galloylquinic acid; brevifolin carboxylic acid	Pomegranate;[7] tea[11]
Hydroxycinnamic acids	Caffeic acid (tentative)	Grape[6]
	Rosmarinic acid	Sage[13]
Ellagitannins and ellagic acid derivatives	Chlorogenic acid (caffeoylquinic acid); dicaffeoylquinic acid	Artemisia annua;[12] Inula viscosa[5]
	Ellagic acid	Pomegranate;[7] guava[10]
	3,4,3′-Tri-O-methylellagic acid; corilagin; granatin B; galloyl-HHDP-hexoside	Pomegranate[7]

Two features of Table 3 are noteworthy. First, quercetin- and kaempferol-type flavonol glycosides recur across botanically unrelated families (Vitaceae, Myrtaceae, Theaceae, Urticaceae), suggesting that this scaffold is a particularly common and effective heme-binding motif rather than a family-specific curiosity. Second, several sources contribute chemically distinctive classes not seen elsewhere in the dataset - curcuminoids in turmeric, abietane diterpenes (carnosol/carnosic acid) in sage, and ellagitannins (corilagin, granatin B) in pomegranate - each of which nonetheless achieved potent β-hematin inhibition, indicating that multiple, structurally unrelated pharmacophores can converge on the same target.

Mechanistic insights and structure–activity relationships

Across all ten studies, the focus was on a common mechanistic hypothesis, consistent with the general model shown in Scheme 1: polyphenolic and related phytochemicals inhibit β-hematin/hemozoin formation by forming a stable, non-covalent complex with free ferriheme that competes with, or physically blocks, heme–heme dimerization and crystal growth.[3–5,7–9,11,13,14] Two non-covalent forces are repeatedly invoked. The primary interaction is hydrogen bonding between the free propionate carboxylate side chains of heme and the hydroxyl and/or carbonyl groups of the inhibitor - most effectively the ortho-dihydroxy (catechol) B-ring and 3-hydroxy-4-keto (hydroxychromone) C-ring motifs of flavonols and flavones.[7,9,11,13] The secondary interaction is hydrophobic π–π stacking between the flavonoid/polyphenol aromatic rings and the porphyrin macrocycle of heme, which is proposed to further stabilize the heme–inhibitor complex and sterically occlude the crystal growth face.[7,9,11,13,14]

Several structure–activity patterns emerge from comparing the individual compounds tested. Among the flavonol isomers morin and quercetin - which differ only in the substitution pattern of the B-ring (a resorcinol-type 2′,4′-diOH arrangement in morin versus a catechol-type 3′,4′-diOH arrangement in quercetin) - morin was consistently the more potent β-hematin inhibitor, remaining active down to 0.04 mg/mL, whereas quercetin required a somewhat higher concentration for comparable inhibition.[9] This indicates that the B-ring hydroxylation pattern, not simply the number of hydroxyl groups, determines the geometry and strength of the heme complex. Glycosylation appears to modulate rather than abolish activity: quercitrin (quercetin-3-O-rhamnoside) and catechin (a flavan-3-ol lacking the 4-keto/2,3-ene C-ring of flavonols) both retained meaningful β-hematin inhibitory activity, but neither matched morin, suggesting that the planar, fully conjugated 3-hydroxyflavone chromophore of the aglycone contributes to optimal heme binding and that bulky 3-O-glycosylation, while not eliminating activity, provides a less optimal steric fit.[6,9] Consistent with this, gallic acid - a simple trihydroxybenzoic acid possessing only a single aromatic ring with three hydroxyls and no fused pyranone/chromone system - was essentially inactive in several studies even at concentrations an order of magnitude higher than active flavonoids, implying that a single phenolic ring furnishes insufficient hydrogen-bond geometry and π-stacking surface to form a productive complex with heme.[7,9,11]

The tea and sage datasets further reinforce a dose–response relationship between the degree of hydroxylation/oxidation of the flavan-3-ol/flavonoid scaffold and activity: the more extensively galloylated and oxidized black-tea catechins (EGCG, ECG, theaflavin, procyanidin B1) were considerably more heme-reactive than the corresponding green-tea catechin pool under plain aqueous extraction, and this gap could be closed only by chemically encouraging catechin autooxidation with sodium bicarbonate.[11] Similarly, in wild sage the presence of multiple hydroxylated diterpenes (carnosol, carnosic acid) alongside flavone and hydroxycinnamic-acid glycosides was proposed to act through the same two-force (hydrogen-bond/π-stack) mechanism, and the crude, multi-component extract again outperformed what would be predicted from any single constituent in isolation.[13] Finally, the marked discrepancy between curcumin as a pure standard (highly active) and crude turmeric extract (roughly ten-fold weaker) is a useful cautionary counter-example: it shows that co-extracted, non-heme-reactive plant material can dilute rather than potentiate an otherwise potent single pharmacophore, so that the "crude extract is more active than isolate" generalization drawn from *Inula viscosa*, pomegranate and grape does not hold universally and must be evaluated case by case.[5,7,8]

Challenges, limitations and future perspectives

Despite the internal consistency of this body of work, several limitations constrain the translational value of the findings and should guide future research.

Single-program scope: As all ten studies derive from a single in vitro protocol, the findings should be interpreted as specific to that body of work rather than as evidence of a generalizable phenomenon in plant-based hemozoin inhibition. Elsewhere in this review, literature on hemozoin inhibition and natural antimalarial compounds[17–22] is used to situate these findings within the broader field.

Assay-level limitations: The β-hematin inhibition assay is an acellular, biochemical surrogate for a cellular and, ultimately, an in vivo process. None of the ten reviewed studies confirmed activity in a *Plasmodium falciparum* culture assay (e.g., SYBR Green or [3H]-hypoxanthine incorporation) or in an animal infection model, so IC50 values against live parasites, and hence a true measure of pharmacological potency, remain unknown for every extract and isolate discussed.[5–14]

Heterogeneous reporting units: The use of percentage dilution and mg/mL interchangeably across studies makes direct, quantitative cross-study potency comparison difficult and necessitates the qualitative, CQ-relative ranking approach adopted rather than a strict numerical ranking.

Compound identification: Except for a handful of pure analytical standards (curcumin, morin, quercetin, catechin, quercitrin), compound identities were assigned from accurate mass and UV-Vis data alone, without confirmatory NMR or authentic standard co-elution; several "unknown" peaks were reported (e.g., in the tea and grape datasets), and isomeric assignments (e.g., among quercetin mono- and di-glycosides) should be regarded as provisional.

Absence of toxicological and selectivity data: None of the ten studies included a cytotoxicity assay against mammalian cells, so a selectivity index (mammalian cytotoxicity relative to antiplasmodial or β-hematin-inhibitory potency) cannot currently be calculated for any of the compounds identified; abietane diterpenes, curcuminoids and tannins in particular have documented pharmacological activities in other contexts that warrant safety evaluation before any therapeutic extrapolation.

Limited mechanistic and structural confirmation: The hydrogen-bonding/π-stacking model of the heme-polyphenol complex is inferred from chemical reasoning and analogy to chloroquine rather than established by molecular modelling, docking, isothermal titration calorimetry or X-ray/spectroscopic characterization of an isolated complex, as several of the primary papers themselves acknowledge;[7,9,11,13] independent structural and kinetic work on heme–inhibitor complexation supports the plausibility of this model but has not been applied directly to the compounds reviewed here.[19,20]

Conclusions

This comparative review of ten in vitro studies, spanning a decade of coordinated research on the β-hematin inhibition assay, demonstrates that natural extracts and isolated phytochemicals from botanically and phytochemically diverse sources - *Artemisia annua*, *Inula viscosa*, grape, pomegranate, turmeric, dietary flavonoids, guava, tea, wild sage and *dwarf nettle* - can inhibit hemozoin biomineralization with potency approaching, matching, or in several cases (pomegranate leaf, black grape peel, *Inula viscosa* crude extract, sage bicarbonate extract) exceeding that of chloroquine under standardized in vitro conditions. The consolidated evidence supports a shared mechanistic model in which polyphenolic hydroxyl/carbonyl groups hydrogen-bond to the propionate side chains of free heme while aromatic rings engage the porphyrin macrocycle through π–π stacking, together capping the hemozoin crystal growth face; this model is consistent across chemically distinct pharmacophores (flavonols, catechins, curcuminoids, abietane diterpenes, ellagitannins), suggesting convergent evolution of heme-binding chemotypes across the plant kingdom rather than a single privileged scaffold.

Ranking the ten sources reveals that whole or minimally processed extracts - particularly aqueous or ethanolic infusions amenable to low-cost, community-level preparation - frequently rival or surpass their own purified fractions, most clearly for *Inula viscosa*, pomegranate and grape, implicating synergistic phytochemical interactions; the curcumin/turmeric pair, however, shows that this pattern is not universal and that co-extracted material can equally dilute a potent single isolate. The sodium bicarbonate extraction studies on sage and green tea provide a particularly promising, low-technology lead for further validation: simple alkalinization of a traditional herbal infusion can convert a weak or inactive preparation into one comparable to chloroquine and can markedly extend the concentration range and storage stability over which activity is retained.

Taken together, these findings are a reminder that natural products remain a real and workable source of antimalarial leads, at a time when resistance to almost every antimalarial drug we currently use threatens to erode two decades of progress against the disease, a picture consistent with independent recent surveys of the plant-derived antimalarial drug-discovery landscape.[21,22] However, translating this reservoir of in vitro activity into validated drug candidates will require the field to move decisively beyond the β-hematin assay: confirmatory testing in live-parasite culture and animal models, rigorous and standardized potency reporting, structural confirmation of the recurring marker compounds, systematic toxicity/selectivity profiling, and direct tests of the synergy and heme-binding hypotheses proposed here. Until such data exist, no dietary, herbal or adjunct-therapy recommendation can be drawn from the findings summarized here.

Acknowledgments

None.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

None.

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