Chapter 10

Approaches, Challenges and Prospects of Antimalarial Drug Discovery from Plant Sources

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Additional information is available at the end of the chapter

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Abstract

Nearly 3.3 billion people globally are at risk of malaria, with 1.2 billion being at high risk. Children under 5 years of age and pregnant women in sub-Saharan Africa still account for a higher percentage of malaria-related mortalities, despite recent reports of decline in malaria mortalities in Africa. Majority of these deaths are caused by *Plasmodium falciparum*, a lethal malaria parasite which has developed resistance to different classes of antimalarial drugs and is responsible for complicated, severe disease. To forestall the debilitating impact of the disease and provide safe and effective alternative therapies, medicinal plants have been explored as a source of new antimalarials. The isolation of quinine and artemisinin from plants present medicinal plants as a robust source of effective antimalarials. In this chapter, we review the different approaches employed in antimalarial discovery from plants, different classes of plant antimalarial compounds and their proposed mechanisms of action. Compounds that show potential for further development based on their high efficacy and selectivity are also highlighted. Common obstacles encountered in the process of antimalarial drug discovery from plant sources are identified and prospects for the identification of new, effective antimalarial components from plant sources are also discussed.

Keywords: Antiplasmodial screening, Antimalarial, Medicinal plants, *Plasmodium falciparum*, Selectivity

1. Introduction

Malaria has remained a leading cause of mortality in close to 100 countries where nearly 2.4 billion people reside, almost half of whom are located in sub-Saharan Africa. With continuous malaria transmission all year round and increasing rates of human movement

in disease-endemic areas, a high burden of antimalarial use in these areas has contributed to global malaria burden [1]. Exposure of parasites to suboptimal antimalarial drug concentrations favors the selection of parasites with traits that enable them to survive in the presence of the drug [2]. Over time, the most lethal strain of the malaria parasite, *Plasmodium falciparum*, has developed resistance to many classes of antimalarial drugs and this contributes to the development of severe malaria complications which can be fatal without prompt treatment. Other less lethal strains, *P. ovale* and *P. vivax*, can exist as latent hypnozoites in the liver which can initiate a relapse months to years after the initial infection [3]. With increased global warming, it has been predicted that the geographic area covered by the malaria vector, *Anopheles* will increase species and this will cause wider transmission of malaria parasites that are resistant to most of the antimalarials presently available [4]. Thus, it has become necessary to develop versatile, robust alternatives to current antimalarial regimens that would be effective against all malaria parasite strains. Within the last decade, efforts have been made to identify new strategies to prevent and treat malaria [5]. Plants have been identified as a robust source for antimalarial drug discovery and interestingly, cinchona alkaloids (such as quinine and quinidine) and artemisinin obtained from plants are still clinically relevant today for the treatment of severe malaria [6].

In order to fast-track the development of effective, alternative medicines from medicinal plants, appropriate pre-clinical studies that confirm their safety and efficacy are required to provide sound experimental data that establish an evidence base. The development of effective medicines from plants is not without its challenges and efforts should be made to address these especially with novel approaches to preclinical screening and clinical testing. Conventional drug development is very time dependent and cost dependent but is rarely rewarding eventually; as the number of approvals for new drugs has declined in recent years [7]. Hence, we also explore the revisited "reverse pharmacology" paradigm to address this problem and secure the future of antimalarial drug discovery.

1.1. Current status of drug discovery from plant sources

Many medicines used against different diseases including cancer, diabetes, hypertension, neurodegenerative disorders and infectious diseases have been sourced from a plant or designed based on scaffolds of compounds isolated from plants. The latest of these include artemether (antimalarial), galantamine (for Alzheimer's disease), nitisinone (for tyrosine-associated metabolic disorder) and tiotropium (anticholinergic), which have all recently been introduced in the United States or are currently involved in late-phase clinical trials [8]. Drug discovery from medicinal plants involves a multi-thronged approach that includes, but is not limited to traditional medicine practitioners, botanists, medicinal chemists, pharmacologists and molecular biologists. Conventionally, plants are selected either randomly or based on their claimed historical medicinal relevance and subjected to sequential extraction and purification steps. This can be very tedious and time-consuming and more effective methods for identifying new lead molecules from plants have been explored. These include chemoinformatics and bioinformatics as tools for in silico drug discovery [9], systems/polypharmacology approach which integrates oral bioavailability tests, druggability, blood-brain barrier permeation, target

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identification and network analysis owing to the complex composition of medicinal plant extracts and their diverse physiological effects [10]. High throughput pharmacological screens and genetic manipulation have also been applied to discover new drug leads from plants, in which plants extracts are screened against an array of receptors with or without gene manipulation and compared to existing drugs [11].

2. Approaches in antimalarial drug discovery

Six major approaches to antimalarial drug discovery have been identified and reviewed, including the investigation of natural products [12]. A plant-based approach is particularly useful in resource-poor, malaria-endemic areas where nearly one-fifth of patients rely on herbal remedies to treat malaria and febrile illnesses [13]. The choice of plants for antimalarial drug discovery may be based on both random and empirical methods to explore biodiversity or through studies guided by traditional use of the plant in the treatment of fever. The latter ethnopharmacological approach has been recognized to give higher success rates for finding active compounds, as over 50% of extracts from ethnomedicinal plants were active in vivo and/or in vitro [14].

2.1. Ethnopharmacology-based plant selection and extraction

Herbal medicines have played a pivotal role in health and disease management for many centuries. Different ancient civilizations, including Mesopotamian, Indian ayurveda, ancient traditional Chinese medicine and Greek unani medicine, show documented evidence for the use of herbs in the treatment of different ailments. In Africa, knowledge of traditional medicine constitutes part of a wholistic system, passed through generations by oral communication and indigenous practices [15]. The scientific exploitation of herbs used ethnomedicinally for pain relief, wound healing and abolishing fevers has resulted in the identification of a wide range of compounds that have been developed as new therapeutics [16].

The major role of ethnopharmacology is to discover new plant-derived compounds based on the traditional use of medicinal plants. The knowledge on the use of plants for fevers and other symptoms of malaria is used to guide the selection of plants to be subjected to antimalarial screening and isolation of active constituents. This is a favored and conservative approach in drug discovery as historical use of a plant as medicine increases the possibility that safe and pharmacologically active compounds would be isolated from it.

2.2. Preclinical efficacy studies

2.2.1. In vitro assays

In vitro cultures of asexual forms of *P. falciparum* are generally maintained in leukocyte-free erythrocytes at 2–5% hematocrit, in Roswell Park Memorial Institute (RPMI) culture medium supplemented with 5–10% human serum at 37°C under reduced oxygen conditions [17]. Advantages associated with this assay are the small amount of test sample required and its

flexibility, as it has been adapted for high — throughput screening of large compound libraries. With latest developments in image processing and automation technology, screening against live parasites in host cells can also be run in 1536-well formats. Also, concentrations producing 50% inhibition (IC_{50}) and 90% inhibition (IC_{90}) can easily be obtained from drug-response curves by nonlinear regression. Drawbacks to these assays include the need for continuous parasite culture and more importantly, the exclusion of host in vivo factors which affect drug disposition and action.

Detection of parasite growth in in vitro assays generally involves the examination of Giemsa-stained smears for viable parasites. This method is very time-consuming, lacks precision and limits rapid, large-scale screening of compounds. Colorimetric determination of parasite lactate dehydrogenase in the presence of nitro blue tetrazolium which is reduced to a formazan derivative has been developed and used successfully [18]. Other methods have been developed which rely on incorporation of radiolabeled metabolic precursors, measurement of dye-stained parasite DNA by fluorimetry or flow cytometry and use of luminometry for genetically modified parasites that express luciferase [19–21]. Fluorescence-based assays that employ DNA-binding fluorophores have also been described, for example, the fluorimetric method described by Benoît et al. [20] in which parasite growth is quantified by stained DNA of viable parasites. Enzyme-linked immunosorbent assays (ELISAs) with monoclonal antibodies which measure *P. falciparum*–specific antigen histidine-rich protein 2 (HRP2) or *Plasmodium* lactate dehydrogenase (pLDH) protein as index of parasite growth have also been reported [22].

Culture conditions for other human and nonhuman *Plasmodium* species are reviewed in detail elsewhere [23]. The culturing of exoerythrocytic sporozoites was elucidated by infecting a primary culture of human hepatocytes with *P. falciparum* and *P. vivax* sporozoites [24]. Though promising, this assay is rarely used as production of large number of sporozoites in insects is a rate-limiting step. Only few in vitro assays have been developed for hypnozoites of *P. vivax* and *P. ovale* and the monkey malaria parasite *P. cynomolgi* [25].

2.2.2. In vivo assays

Mouse models of malaria infection using rodent parasites are especially useful for studying the pathological effects of interactions between the host and the parasite. These models predict clinical outcomes of infections such as parasitemia, sequestration of parasitized red blood cells, splenomegaly, pulmonary edema and hematological and biochemical phenomena. Laboratory rodent parasites such as *P. berghei* and *P. yoelii* are used for evaluation of plant extracts and compounds [26]. With advances in genetic manipulation, humanized mouse models to study blood- and liver-stage *P. falciparum* infections in genetically modified mice have been recently reported [27]. These models have also been used in studying cell-mediated immune responses to liver-stage malaria vaccines [28]. Preliminary tests against *P. vivax* in non-human primate models like *Aotus* and *Saimiri* monkeys have also been carried out [29].

2.2.3. Bioactivity-guided studies, compound isolation and identification

In common practice, traditional herbal remedies are prepared in water, either at room temperature or by boiling to obtain a decoction. Alcoholic solvents are also used as they produce higher extract yield and extract a wider variety of chemical components compared to aqueous extraction [30]. Separation and purification processes for antimalarial plant extracts and fractions involve different chromatographic methods. Frequently, as the extract is separated sequentially, antiplasmodial activity is monitored with a high-throughput in vitro bioassay until the compounds responsible for activity are isolated. This method is based on the assumption that antiplasmodial activity is limited to one or few compounds, whereas when such activity is due to different compounds acting synergistically, it may be lost with further separation [31]. Chromatographic procedures commonly employed include flash column, medium- and high-pressure liquid chromatography and centrifugal countercurrent chromatography. The structure of isolated compounds is determined on the basis of their spectroscopic properties using mass spectrometry, ultraviolet and infrared spectroscopy and complete proton and carbon mapping using one- and two-dimensional nuclear magnetic resonance techniques. It has also been possible to use tandem or hyphenated techniques of these spectroscopies for full stereochemical elucidation of constituents without isolation from extracts [32]. The compound obtained is thereafter subjected to further testing, extending to transmission and radical cure assays. Following the selection of a lead compound, it may be optimized by synthesizing chemical derivatives with the desired bioavailability, potency and selectivity before pre-clinical testing for efficacy and safety, preparatory for phase I clinical testing [33].

3. Isolated compounds and antiplasmodial activity

Some examples of identified compounds that exhibit good antimalarial activity *in vitro* are shown in **Table 1**. Criteria adopted for selection of the compounds shown were inhibition of *P. falciparum* growth by 50% at a concentration of either < 5 μg/mL or < 5 μM in vitro, with high selectivity (>100) for the parasite, where selectivity is expressed as

$$Selectivity\left(S\right)=\frac{\mathrm{EC}_{50}}{\mathrm{IC}_{50}} \quad (1)$$

where EC_{50} = effective concentration required to inhibit cellular growth by 50% and IC_{50} = concentration required to inhibit parasite growth by 50%.

From the compounds shown in **Table 1**, it is evident that a remarkable diversity of plant-derived compounds exists and they can form good templates for the design of novel antimalarials. One example of such is gedunin, a limonoid extracted from the leaves of *Azadirachta indica* with high antiplasmodial activity in vitro. Its antiplasmodial potency was attributed to the α,β-unsaturated ketone group in Ring A of its limonoid backbone, a 7α-acetate group as

well as its furan ring [34]. *Dichroa febrifuga* is a popular fever remedy in traditional Chinese medicine and guided studies led to the isolation and identification of febrifugine over 50 years ago [35]. Studies on febrifugine were hindered for a long time because of its toxicity. Some of its derivatives with good antiplasmodial activity have however been shown to exhibit lower toxicity compared with the parent compound [35]. The monoterpene indole alkaloid ellipticine and its isomer olivacine isolated from the bark of *Aspidosperma olivaceum* were shown to possess antiplasmodial effects in addition to its previously reported antitumor properties [36]. The selectivity of the ellipticine and olivacine for the parasitic targets was evident from the high *S* values (500–1200, ellipticine and 330–390, olivacine) against *P. falciparum* K1 and 3D7 [36]. Ellipticine was shown to be antiplasmodial, by inhibiting heme crystal growth and interacting with parasite DNA. It was speculated that this effect was similar to that of other analogous cryptolepine- and harmane-type indole alkaloids, depending on structural similarity [36]. *Uvaria leptocladon* is a shrub growing in the West Usambara Mountains of Tanzania, where it is used against cerebral malaria [37]. Investigation of the Tanzanian *U. leptocladon* root bark afforded the isolation of the chalcones, uvaretin and diuvaretin, which were shown to possess antiplasmodial activity with $IC_{50} < 5$ μg/mL [38]. Antiplasmodial screening of *Dorstenia barteri* twigs yielded the isolation of the prenylated chalcones, bartericin A (IC_{50} = 2.15 μM) and 4-hydroxylonchocarpin (IC_{50} = 3.36 μM) which were devoid of toxicity to erythrocytes at concentrations below 20 mM [39]. The authors deduced that the presence of a hydroxylated prenyl group on carbon 5′ on Ring B of bartericin A enhanced its antiplasmodial activity compared to a prenyl group at the same position in 4-hydroxylonchocarpin [39]. Lanaroflavone is a biflavonoid isolated from the methanol extract of the aerial part of *Campnosperma panamense* Standl. In vitro screening revealed its highly selective (*S* = 159) antiplasmodial activity (IC_{50} = 0.48 μM) [40]. For this compound, it was observed that its C-4‴—O—C-8 interflavonoid linkage was relevant for antiplasmodial activity [40].

A study of species of *Carpesium* genus used as traditional remedies for the treatment of parasite infections led to the identification and isolation of ineupatorolide A, a sesquiterpene from *Carpesium rosulatum*. Of particular interest were its high antiplasmodial activity (IC_{50} = 0.019 μM) and selectivity (*S* > 1000) [41]. *Bowdichia nitida* Spruce ex Benth., commonly referred to as "sucupira," is distributed in the Brazilian Amazon and the seeds of this plant are traditionally used for rheumatic arthritis, fever and gouty arthritis [42]. 6α,7β-Diacetoxyvouacapane isolated from a methanol extract of *B. nitida* seeds displayed high activity against *P. falciparum* 3D7 (IC_{50} 0.39 μg/mL) and high selectivity for the parasite, as cytotoxic IC_{50} on COLO 201 cells was higher than 100 μg/mL [42]. Another antiplasmodial compound identified as neosergeolide, a quassinoid obtained from the root and stem of *Picralemma sprucei*, had high antiplasmodial activity (0.002 μM) and was cytotoxic toward selected tumor cell lines at concentrations ranging from 5 to 27 mg/mL [43]. Antiplasmodial activity-aided fractionation of *Piptadenia pervillei* leaves afforded the identification of (+)-catechin-3-gallate and (+)-catechin-5-gallate, which displayed high antiplasmodial effects against *P. falciparum* FcB1 and had no significant cytotoxic effects against the human embryonic lung cells MRC-5 [44].

Other compounds have also been investigated and found highly active against hepatic stage *Plasmodium* species. N-Cyclopentyl-tazopsine, a semisynthetic derivative of a plant-derived

morphinan compound, tazopsine, was shown to have specific activity against liver-stage parasites of *P. falciparum* (IC_{50} = 42.4 μM, *S* = 60) and *P. yoelii* (IC_{50} = 3.3 μM, *S* = 46) [45]. Its efficacy against hepatic-stage parasites indicates its potential for development as a prophylactic agent.

Compounds	Plant source	Family	Antiplasmodial activity	Source
Gedunin	*Azadirachta indica*	Meliaceae	(Pf D6) 0.039 μg/mL (Pf W2) 0.02 μg/mL	[34]
Febrifugine	*Dichroa febrifuga*	Hydrangeaceae	(Pf W2) 0.53 ng/mL (Pf D6) 0.34 ng/mL	[35]
Ellipticine	*Aspidosperma vargasii*	Apocynaceae	(Pf K1) 0.81 μM (Pf 3D7) 0.35 μM	[36]
Olivacine	*Aspidosperma olivaceum*	Apocynaceae	(Pf K1) 1.4 μM (Pf 3D7) 1.2 μM	[36]
Uvaretin	*Uvaria* spp.	Annonaceae	(Pf K1) 3.49 μg/mL	[37, 38]
Diuvaretin			4.20 μg/mL	
Bartericin A	*Dorstenia barteri*	Moraceae	(Pf W2) 2.15 μM	[39]
4-Hydroxylonchocarpin			3.36 μM	
Lanaroflavone	*Campnosperma panamense*	Anacardiaceae	(Pf K1) 0.48 μM	[40]
Ineupatorolide A	*Carpesium rosulatum*	Asteraceae	(Pf D10) 0.019 μM	[41]
6α,7β-Diacetoxyvouacapane	*Bowdichia nitida*	Leguminosae	(Pf 3D7) 0.97 μM	[42]
Neosergeolide	*Picralima sprucei*	Simaroubaceae	(Pf K1) 0.002 μM	[43]
(+)-Catechin-3-gallate	*Piptadenia pervillei*	Fabaceae	(Pf FcB1) 1 μM	[44]
(+)-Catechin-5-gallate			1.2 μM	

Table 1. Antiplasmodial compounds with high selectivity isolated from plants.

4. Isolated compound classes and intra-parasitic targets

4.1. Alkaloids

This group of plant secondary metabolites represents the largest group of plant secondary metabolites with the highest number of compounds displaying potent antiplasmodial activity. They also serve as good templates for synthesis of many quinolone-based antimalarial drugs. Alkaloids displaying potent antiplasmodial activity occur as steroidal alkaloids, bisbenzylisoquinolines, naphthylisoquinolines, indoloquinolines and indolomonoterpenoid alkaloids, among others. Quinoline alkaloids isolated from the bark of *Cinchona officinalis* including

quinine, quinidine, cinchonine and cinchonidine (**Figure 1**) are all highly effective against malaria, with cure rates exceeding 98% in humans [33]. Although quinine is associated with serious side effects, it has remained an important drug to treat severe malaria due to chloroquine resistance. Quinine is schizonticidal against all intraerythrocytic malaria parasites and gametocytocidal for *P. vivax* and *P. malariae*, but not against *P. falciparum* gametocytes [46].

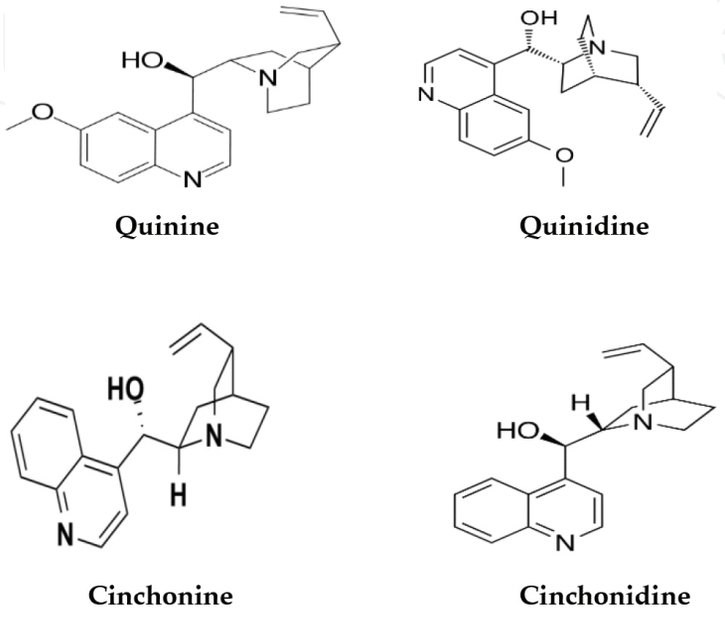

Figure 1. Chemical structures of cinchona alkaloids: quinine, quinidine, cinchonine and cinchonidine.

Although the mechanism of action of quinine has not been fully resolved, it has been reported to exhibit inhibitory effects on heme polymerization and heme catalase activity [47]. Following the success of quinine identification and use, natural antiplasmodial alkaloids have been isolated and reviewed by Kaur et al. [48]. Some alkaloids have been reported to inhibit fatty acid biosynthesis in the parasite [22], while some act as resistance reversers. The monoindole alkaloids strychnobrasiline and malagashanine isolated from Strychnos myrtoides potentiated the effects of chloroquine, quinolones, aminoacridines and halofantrine [49]. Malagashanine has no intrinsic antiplasmodial or cytotoxic action, but aids chloroquine accumulation in drug-resistant parasites by improving chloroquine influx and preventing its efflux from the parasites [50].

4.2. Flavonoids and chalcones

Flavonoids occur ubiquitously in many higher plants where they act as growth regulators and offer protection against plant pathogens [51]. They have been proposed to act by inhibiting the fatty acid biosynthesis (FAS II) pathway, which is present in the parasite's apicoplast but absent in human hosts [52]. The flavonoid, luteolin-7-O-β-D-glucopyranoside, was reported as the first natural product that targets plasmodial FAB I enzyme which regulates the FAS II pathway [53]. Some flavonoids have also been shown to inhibit L-glutamine and myoinositol influx

into infected erythrocytes or act by interfering with hemin degradation [54, 55]. In addition, chalcones have been proposed to act by inhibiting cyclin-dependent protein kinases and plasmepsin II [56].

4.3. Terpenes and terpenoids

In recent times, attention has been devoted to this class of compounds especially sesquiterpenoids, following the discovery of the endoperoxide sesquiterpene lactone; artemisinin. These compounds are attractive because some possess intrinsic antiplasmodial activity and offer good starting points for chemical modification into derivatives with desired physicochemical properties and enhanced efficacy. Artemisinin and its derivatives owe their antiplasmodial effects to the presence of an endoperoxide bridge that generates toxic-free radicals when it is broken down (**Figure 2**). Another example of a highly potent antiplasmodial sesquiterpene is ineupatorolide A (**Table 1**).

Apart from the major classes of isolated compounds discussed above, other examples such as xanthones, stilbenes, coumarins, lignans, tannins and steroids have also been reported to exhibit potent antiplasmodial effects [57].

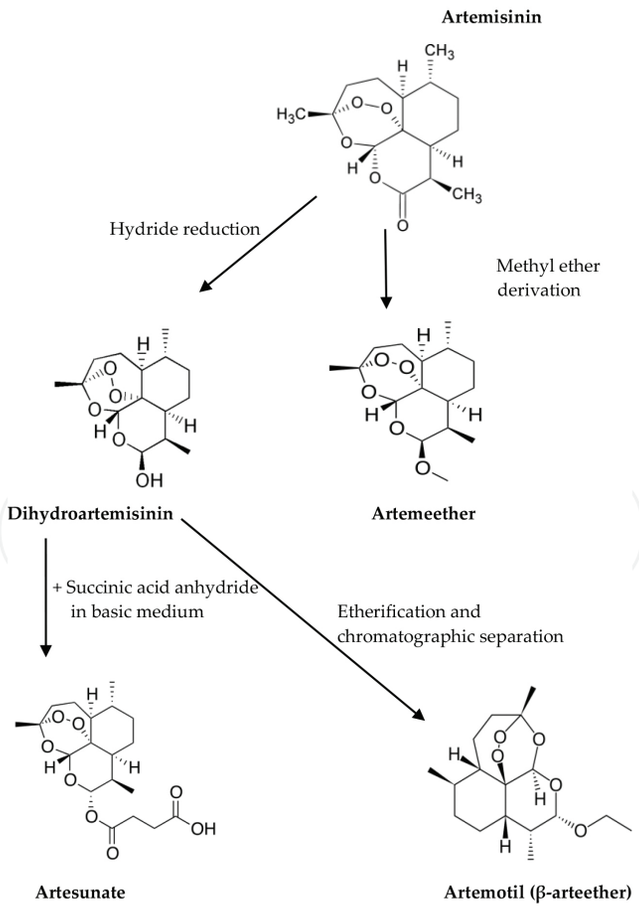

Figure 2. Artemisinin and its chemical derivatives in clinical use.

5. Clinical studies

Literature search revealed only few plant-derived extracts or compounds undergoing clinical studies and these are shown in **Table 2**. Spirotetrahydro β-carbolines (spiroindolones) present a unique group of compounds that share structural similarities with strictosamide, an iridoid indole alkaloid identified in an extract of *Nauclea pobeguinii* stem bark, but also present in *Nauclea latifolia* and *Nauclea officinalis* extracts [58, 59]. A spiroindolone (NITD609, **Figure 3**) displayed low IC_{50} within the range of 0.5–1.4 nM, showed no evidence of diminished potency against drug-resistant strains and was not significantly cytotoxic to mammalian cells [60, 61]. It was also effective against clinical isolates of *P. falciparum* and *P. vivax* (IC_{50} < 10nM) and comparable to artesunate. Additionally, it inhibited gametocyte development in vitro and oocyst development in mosquitoes [60]. *Argemone mexicana* decoction administered orally also produced antimalarial effects that were comparable to artesunate-amodiaquine combination in patients [62].

Plant extract/compound	Stage of clinical development	Mechanism of action
Nauclea pobeguinii [58]	Phase IIb/III	Not known
Argemone mexicana [62]	Phase IIb/III	Not known
NITD609 [60, 61]	Phase IIa	Chemotherapeutic, transmission blocking

Table 2. Plant extracts and natural product–derived compound in clinical development.

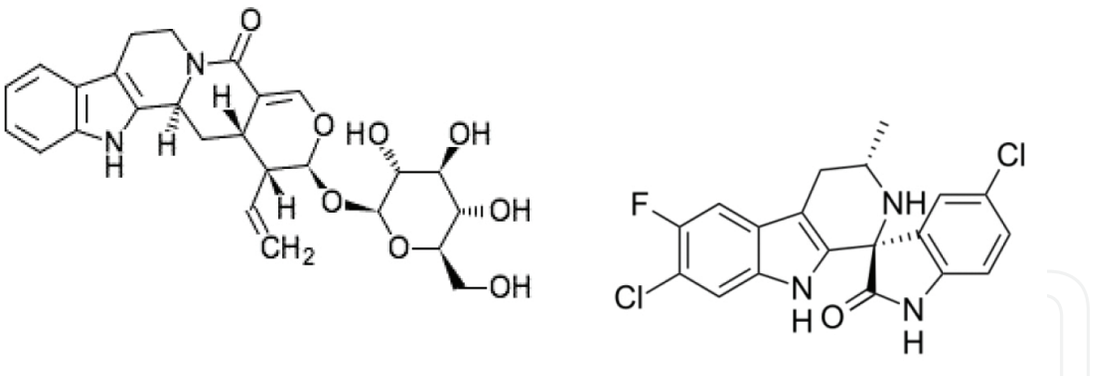

Figure 3. Chemical structures of strictosamine, an iridoid indole alkaloid and structurally similar spiroindolone in clinical development, NITD609.

6. Reverse pharmacology: from bedside to bench

Conventional drug discovery and development is an energy-, time- and resource-demanding venture; hence, the entire process results in minimal success rates. Millions of compounds are

involved during initial screening and identified hits are ranked based on potency, ease of synthesis, known limitations to therapeutic use and novelty to determine a possible lead compound [33]. The lead compound is thereafter subjected to preclinical tests and various optimization processes to confer desired chemical and pharmacokinetic properties on it before final clinical testing. After passing through rigorous Phases I-III trials, it may be accorded statutory approval for clinical use. This is very expensive and time-consuming and many pharmaceutical companies are looking for new approaches in drug discovery that will lead to expedited launch of new, effective and safe drugs.

Reverse pharmacology is a science that integrates well-documented clinical experiences and observations toward lead development, through interdisciplinary studies (preclinical, clinical) for drug development [63]. Here, "safety" is the starting point as well-documented evidence of traditional use as medicine. This provides an important basis for further scientific testing. Hence, reverse pharmacology adopts a "bedside to bench" approach, compared to conventional "bench to bedside" drug discovery and development.

The use of *Artemisia annua* as treatment for fever and malaria in traditional Chinese medicine afforded the discovery of artemisinin through a reverse pharmacology approach. Today, artemisinin derivatives like artemether, artesunate, artemether and dihydroartemisinin remain useful antimalarial agents against drug-sensitive and drug-resistant malaria. However, the case of artemisinin is a unique one where artemisinin was identified as an active molecule, as not all traditional medicinal herbal extracts owe their therapeutic effects to a single chemical entity. The effects of some extracts may be due to different phytochemicals acting on different targets or a synergistic effect between different constituents and further separation and purification may lead to a loss of activity [30]. In this case, bioactivity markers should be identified and a standardized formulation of the extract should be prepared and screened using a systems biology approach before consideration for further development.

An example of antimalarial drug development using the reverse pharmacology approach in recent times is seen in the study of a standardized extract of *A. mexicana* for the treatment of malaria [64]. Initially, the authors conducted a retrospective treatment-outcome study to select a candidate for development before a dose-escalating clinical study to identify and choose a dose with desirable safety and efficacy. Next, they carried out a randomized controlled trial for comparison of the selected phytomedicine with conventional first line antimalarial therapy followed by identification of active compounds which could be employed as chemical markers to standardize the phytomedicine. This scheme was used successfully and can be adopted for antimalarial drug development (**Figure 4**). The process of identifying chemical and/or biological markers of efficacy which are used to ensure herbal medicine quality is known as standardization. This is an important step in drug development from herbal medicines, as quantity and quality of secondary metabolites depend on intrinsic factors, environmental factors and biotic factors [65].

It is interesting to note that subsequent to the report on the clinical efficacy of *A. mexicana* [62], three antiplasmodial protoberine-type alkaloids were isolated by conventional methods from an extract of *A. mexicana*, namely, berberine, allocryptopine and protopine, but berberine was

found to be cytotoxic relative to the parent extract, while allocryptopine and protopine were more selective for parasites compared to berberine [66] (see **Figure 4**).

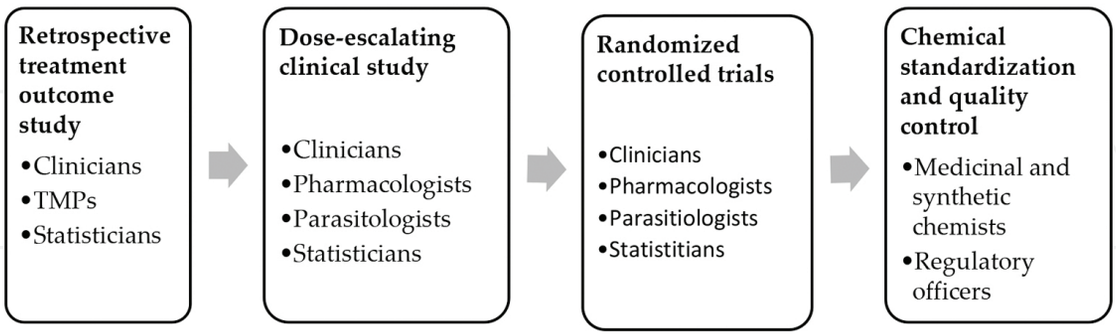

Figure 4. Antimalarial drug development by reverse pharmacology flow chart as described by Willcox et al. [64], showing key personnel involved during each stage of the process.

7. Conclusions

Naturally occurring antiplasmodial compounds in plants show vast chemical diversity but also exist within a complex mixture of other plant secondary metabolites which in itself constitutes a major challenge to efforts in identifying compounds responsible for biological effects. Other problems with plant-based drug discovery process range from the basic ones like sustainable access to plant material, seasonal and environmental variations and legislative issues concerning plant use, to challenges concerning complex fractionation procedures, small quantity of pure compounds and poor pharmacokinetic/physicochemical properties that negatively affect druggability [67]. With an increasing understanding and use of genomics, it is possible that bioactive molecules can be produced more efficiently using plant-cell cultures and genetically modified microbes [68]. This has already been exploited in the production of artemisinin precursors from genetically modified *Saccharomyces cerevisiae* and *Escherichia coli* [69].

Innovative drug discovery through reverse pharmacology or conventional methods especially in resource-constrained remote areas where medicines are urgently needed should be given more attention. There is the need to explore other aspects of the use of plant extracts and compounds as efficacy boosters or drug resistance reversers in combination with conventional therapy [50]. Efficacy screening against the parasite at all stages of development including gametocytes and hypnozoites should be incorporated in preclinical drug testing as they are often overlooked yet useful tools to identify agents that block transmission of resistance and prevent relapse [70]. In the course of literature review, a number of antimalarial compounds reported also displayed significant cytotoxic effects on human cells. Thus, screening for inhibitors against parasite-specific targets in organelles like the apicoplast and pathways such as heme degradation and type II fatty acid biosynthesis would likely identify active leads with highly selective antiplasmodial action.

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