Section 4

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Pharmacology

Exploring Cameroonian Medicinal Plants for a New Generation of Anti-malarial Compounds

Cameroon

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Summary

Malarial drug resistance is the major challenge, sapping control efforts as it affects virtually all drugs available on the market today. At the Biotechnology Unit, University of Buea, the Malarial Drug Discovery and Development Research Group aims to explore the rich Cameroonian flora as a potential source of new antimalarial compounds. This initiative has received support from the Cameroon Government, local partners and international bodies, including the International Programme in Chemical Sciences (IPICS), the International Foundation for Science (IFS) and The World Academy of Sciences (TWAS). Over the past 25 years, we have succeeded in thoroughly documenting more than 200 plant species commonly employed for the treatment of malaria, and establishing antiplasmodial activity in more than 50 of them. Among the plant species tested, *Alastonia bonei, Bersama* spp., *Dacryodes edulis, Guibourtia tessmani, Hypericum lanceolatum, Kigelia africana, Perperomia* spp. and *Tectona grandis*, showed the most promising activity against drug-resistant malaria. More than 70 pure molecules, from Cameroonian medicinal plants, were isolated and tested, showing a wide range of activities. A medium-scale screening platform has been established for malaria including both *in vitro* and *in vivo* models. We plan to develop this platform further to include automated systems with improved screening output, as well as proceeding to more advanced drug development stages including pharmacokinetics and pharmacodynamic profiling of the most promising products identified thus far.

Background and Justification

Malaria has steadily inflicted a heavy socioeconomic burden on the people of Africa and other tropical regions for centuries, despite enormous efforts to control it by different stakeholders both at the local and international levels. For several decades, drug resistance has remained the greatest challenge to malaria control, and is one of the formidable obstacles that foiled earlier attempts at seeing malaria eradicated in the 1970s. The late 1980s witnessed an unprecedented spread of chloroquine-resistant *Plasmodium* strains across Africa. So far, resistance has been well established in three of the five *Plasmodium* species responsible for human malaria (*P. falciparum, P. vivax,* and *P. malariae*), and this concerns virtually all drugs in current use. This enduring challenge underline the urgent need to broaden the repertoire of anti-malarial drugs, especially for the countries where malaria is endemic.

For hundreds of years, plants have constituted the basis of traditional medicine systems

and a plant-derived products have been a major source for drug development (Titanji *et al*., 2008). Quinine and artemisinin are examples of plant-derived drugs that have been used successfully against resistant strains of malarial parasites. In some rural areas of Cameroon, traditional anti-malarial medicines are even preferred to pharmaceutical compound drugs, suggesting that the herbal preparations used by traditional healers might contain useful active ingredients. Most central African countries converge in the Congo basin, which represents the second largest contiguous rainforest in the world after the Amazon. The Congo basin forests hold 20% of the world tropical moist forests and 80% of the tropical moist forests in Africa. In Cameroon, traditional medicine practitioners and communities use more than 500 medicinal plants species in their various remedies including antimalarials (Zofou *et al*., 2014).

The idea of designing novel antimalarial drugs especially from Cameroonian medicinal plants was prompted within our research team in the late 1980s, with the main focus on identifying and evaluating locally-used plant species acclaimed for their efficacy and safety. In order to either standardize these as phytomedicines or exploit them as sources of new drug leads. Thus, for many years, our team has been working closely with traditional healers in Cameroon with the main aim of improving the exploitation of natural resources in handling malaria and other endemic diseases.

The main research objectives of the Biotechnology Unit in the field of malarial drug research include:

- the identification and characterization (*in vitro*) of antimalarial products from selected medicinal plants used to treat malaria in Cameroonian folk medicine;
- the preclinical study of potential drug leads identified through *in vivo* screening for antimalarial activity and toxicity and pharmacokinetics; and
- drug target prediction and validation using bioinformatics tools.

Description

Drug research and development (R&D) is, in general, a long and laborious process requiring substantial funding, good infrastructure, qualified personnel and an enabling political and institutional environment. The starting product, from a range of sources (plants, marine flora and microorganisms) may be chosen and screened at random or the source-materials may be selected based on previous knowledge (ethno-botanical or ethno-pharmacological surveys) of their use in communities. A wide range of technologies is available for the extraction of active components and essential oils from medicinal and aromatic plants. The choice depends on the nature of the source material,

the nature of experimental techniques and models employed as well as cost feasibility and the suitability of the process to the particular context. Crude extracts with promising activity tipically undergo bioassay-guided fractionation towards detection and isolation of potentially active pure ingredients.

The systematic isolation and testing of molecules from plant parts against the various parasites is not a feasible option because of the prohibitive cost of such a vast enterprise. The use of ethno-botanic information to select plants for screening has proved to be a fruitful and cheaper approach for drug discovery. Once identified, active principles can be optimized by chemical synthesis to obtain more active products at affordable costs. The entire pool of molecules present in different parts of the plant under consideration can also be isolated and tested against different parasite types in the search for potentially active compounds (Fig. 1).

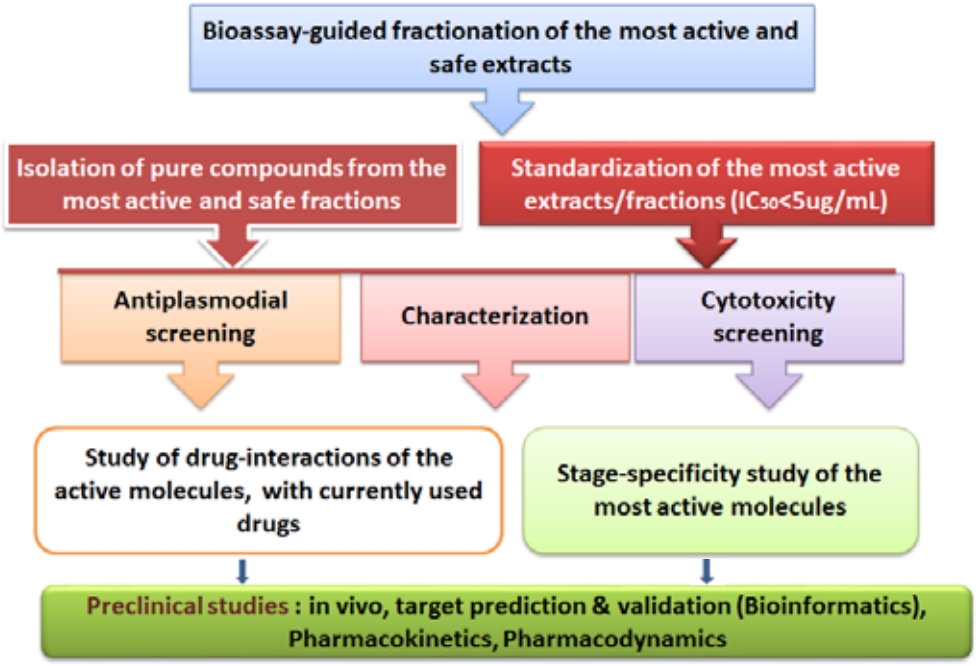

Figure 1: General protocol for malaria drug discovery and development from medicinal plants (Zofou *et al.*, 2011a).

Both chloroquine-sensitive (3D7, D6) and multi-drug-resistant (W2, W2mef, K1 and Dd2) *Plasmodium* strains were kindly donated by BEI-Resources/MR4 (MR4, Manassas, VA, USA), and maintained in continuous culture, with back up stored in liquid nitrogen. In earlier studies, the Vietnamese strains were used (Tantchou *et al*., 1986). Field isolates made up of active parasites collected locally from malaria patients were also used in testing the plants products identified. The *in vitro* screening employs the semi-automated techniques of Desjardins, using both light microscopy (normal light and fluorescence) and the parasite lactate dehydrogenase assay (pLDH). Fig. 2 illustrates a plate result of pLDH-based screening.

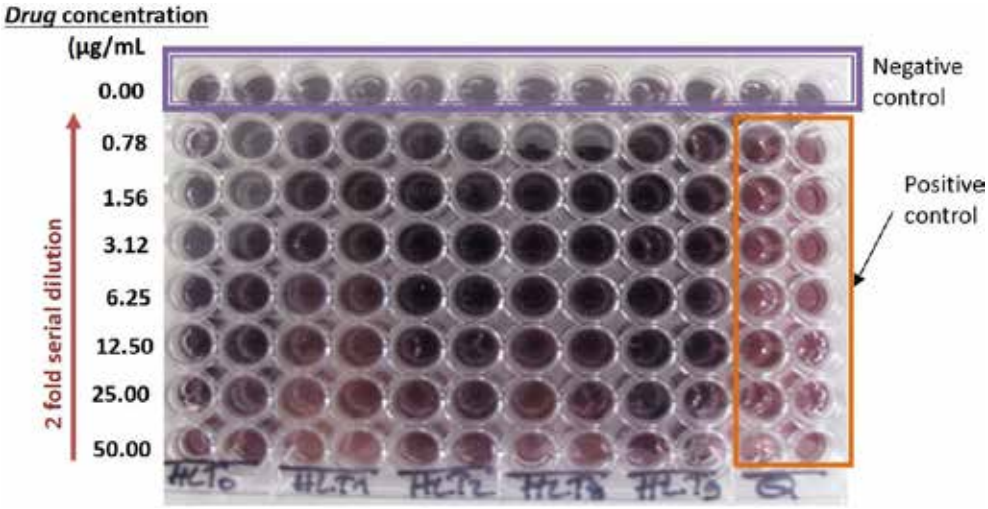

Figure 2: Photograph of the pLDH plate obtained with some compounds of *H. lanceolatum* (Zofou *et al*. 2011a). The experiment was conducted in duplicate and included, from left to right the following compounds: HLT0, HLT1, HLT2, HLT8, HLT9 and QN. The denser the coloration, the higher the parasitaemia in the concerned well.

From the medicinal plants identified as well as reports of species from other parts of Africa, we have screened more than 200 plant species, some of which exhibit very interesting properties in terms of antimalarial activity and safety. From these, 70 compounds (including three newly–discovered ones) were isolated from four plant species, including the edible plant species *Dacryodes edulis*, and their antiplasmodial profile established. Twelve of these 70 compounds that scored the highest ratings were selected and screened following the bioassay-guided fractionation approach. The antimalarial activity was tested alongside with toxicity of the different products against one of the most fragile mammalian cell lines, the LLC-MK2 monkey kidney cell line, to define their relative safety. For the most active pure molecules isolated, we also evaluated their interaction patterns with drugs already in use such as artemether and quinine. Likewise, the activities of the most active and non-toxic candidates were tested against different life cycles in order to have preliminary information on their mechanism of action.

Results

These studies confirmed the antimalarial properties of seven plant species used in Cameroon to treat malaria and other fevers, and five new antimalarial molecules were identified from these, with *Dacryodes edulis* (Zofou *et al*., 2013), *Bersama* spp. (Nondo *et al*., 2015), *Perperomia* spp. (Ngemenya *et al*., 2014), *Tectona grandis* (Kopa *et al*., 2014), *Hypericum lanceolatum* (Zofou et al., 2011a) and *Kigelia africana* (Zofou *et al*., 2011b, 2012) being the most active. These species underwent further purification through bio-assay-guided fractionation to yield several compounds of which 15 were found to be highly active against both chloroquine-sensitive and drug-resistant malaria parasites. Table 1 summarizes the *in vitro* activity of the compounds isolated from *K. africana* while Fig . 3 illustrates their drug-interaction profiles with reference malaria treatments.

Product code	Quantity (mg)	Extraction yield (%)	IC_{50} on W2	IC_{50} on W2mef	IC_{50} on CAM10	$IC_{5}0$ on SHF4
Atranorin	30	0.10	4.41 ± 0.35	1.78 ± 0.18	2.81 ± 1.07	2.78 ± 0.29
KAE3	17	0.06	1.60 ± 0.00	1.86 ± 0.15	2.17 ± 0.55	8.02 ± 0.55
KAE7	103	0.15	1.54 ± 0.00	1.02 ± 0.17	2.34 ± 1.15	2.70 ± 0.29
KAE10	62	0.09	53.84 ± 19.39	12.89 ± 0.87	7.13 ± 3.35	6.71 ± 0.12
CQ	-	-	0.29 ± 0.02	0.26 ± 0.02	0.25 ± 0.04	0.19 ± 0.02
QN	-	-	0.23 ± 0.02	0.27 ± 0.04	-	0.14 ± 0.05
ART	-	-	0.03 ± 0.01	0.04 ± 0.00	-	0.03 ± 0.01

Table 1: Extraction yield and antiplasmodial activity of pure compounds from *Kigelia africana* (Zofou *et al*., 2011b). W2, W2Mef, CAM10 and SHF4 are different strains of *Plasmodium*. CQ = chloroquine, QN = quinine, ART = artemether, three standard antimalarial drugs.

More interestingly some of these compounds showed synergy with artemether and antagonistic effects with quinine, suggesting their potential as partner molecules in new antimalarial combination therapies (ACT) or quinine-based drug combinations against multi-drug-resistant malaria. Further studies including *in vivo* testing of the combinations and the study of their toxicity are required to move forward in the exploration of this plant species as source of new antimalarials.

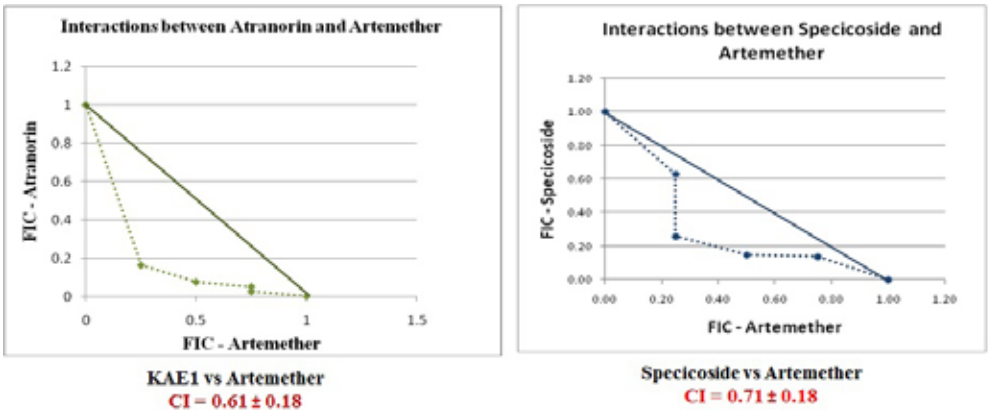

Figure 3: Synergistic effects between selected compounds from *Kigelia africana* and artemether (from Zofou *et al*., 2012).

Partnerships

Over the years, we have succeeded in establishing a mutually-fruitful collaboration between various teams. Our local network in Cameroon is illustrated as shown in Fig. 4 and includes traditional medicine practitioners through the National Order of Tradi-practioners in Cameroon; the Natural Product Laboratory of the University of Dschang; the Phytochemistry Laboratory of the Institute of Medical Research and Studies of Medicinal Plants (IMPM) for phytochemistry work; the Gallenic Pharmacy and Drug formulation team of the IMPM; the Department of Botany and Plant Physiology and the National Herbarium for plant identification; the Department of Sociology of the University of Buea; the Botanic Garden, Limbe; and the National Herbarium, Yaounde'.

A research collaboration has recently been established with the Muhimbili University of Health and Allied Sciences (Dar es Salaam, Tanzania) aimed at fostering the efforts of both institutions as far as drug discovery from African flora is concerned. In this regard, a PhD student has already been trained at the Biotechnology Unit, University of Buea, on malaria culture and screening techniques. From this highly promising partnership, a scientific paper has been published on the documentation and primary investigation of over 100 hundred plants species from Tanzania (Nando *et al.*, 2015).

Figure 4: Collaboration and networking in drug discovery research at the Biotechnology Unit, University of Buea, Cameroon.

Impact

The main impact of our work (see Titanji *et al*., 2008 and Zofou *et al.*, 2014) so far has been to stimulate research in and provide a framework for the scientific study of medicinal plants. Work is now ongoing in various laboratories in the country not only on malaria, but also on filarial diseases, bacterial infections, and even non-communicable conditions like diabetes and obesity.

We have also trained a cohort of researchers in the field of medicinal plant studies including three PhD and more than a dozen MSc degree holders.

Finally, some the active antimalarial compounds discovered in our work are in pre-clinical studies with a view to developing phyotomedicines for the treatment of malaria. Meanwhile, local communities continue to use the plants which we have validated as active and non-toxic for the management of fevers and malaria.

Replicability

The Government of Cameroon, through its Institute of Medical Research and Study of Medicinal Plants has instituted a project for the production of improved phytomedicines for malaria and other diseases. Our group provides screening services and technical advice to this project. The team was recently honoured with the selection of Denis Zofou as the laureate for the 2014 TWAS-ROSSA Young Scientists Prize in Applied Sciences.

It was not our aim to seek patents from our work; rather we sought to scientifically validate herbal medicines for the most common maladies in our setting as a contribution to the fight against diseases of poverty.

Lessons Learned

The main lessons learned are that many of the claims of traditional medicine practitioners can be verified by using simple bio-assays, and the success rate is far higher than if random systematic screening of plants is carried out. Another lesson is that confidence can be built and maintained if the traditional practitioners and community members are certain that they are not being exploited for financial gain.

Future Plans

We plan to collaborate with chemists to scale up the production of the most active compounds discovered by our group and to carry out preclinical and subsequently clinical trials of these compounds for malaria treatment. This is a major undertaking which will need the collaboration of the private sector.

Meanwhile, several research projects funded by the IFS, TWAS and the African Union to develop malaria phytomedicines will continue to serve as a training ground for MSc and PhD students.

We also plan to expand our programme to look for plant-derived remedies for the neglected tropical diseases other than malaria.

Publications

- Titanji VPK, Zofou D and Ngemenya MN (2008). The anti-malarial potential of medicinal plants used for the treatment of malaria in Cameroonian Folk Medicine. African Journal Traditional Complementary and Alternative Medicines, 5 (3): 302- 321.
- Zofou D, Kowa TK, Wabo HK, Ngemenya MN, Tane P and Titanji VPK (2011a). *Hypericum lanceolatum* (Hypericaceae) as a potential source of new antimalarial agents: a bioassay-guided fractionation of the stem bark. Malaria Journal, 10:167. doi:10.1186/1475-2875-10-167.
- Zofou D, Tematio EL, Ntie-Kang F, Tene M, Ngemenya MN, Tane P and Titanji VPK (2013) New antimalarial hits from *Dacryodes edulis* (Burseraceae) - Part I: Isolation, *in vitro* activity, in silico "drug-likeness" and pharmacokinetic profiles. PLoS ONE. 10/2013.
- Zofou D, Tene M, Tane P and Titanji VPK (2012). Antimalarial drug interactions of compounds isolated from *Kigelia africana* (Bignoniaceae) and their synergism with artemether, against the multidrug-resistant W2mef *Plasmodium falciparum* strain. Parasitology Research, 110(2): 539-44. DOI: 10.1007/s00436-011-2519-9.
- Zofou D, Tene M., Ngemenya MN, Tane p. and Titanji VPK (2011b). In vitro antiplasmodial activity and cytotoxicity of extracts of Selected medicinal plants used by traditional healers of Western Cameroon. Malaria Research and Treatment, Article ID 561342, 6 pages.

Reference

- Nando *et al*. (2015). J. Med Plants Res.; 9(1):179-192. DOI:10.5897/JMPR2014.5685.

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