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Plants traditionally prescribed to treat *tazo* (malaria) in the eastern region of Madagascar

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Abstract

Background: Malaria is known as tazo or tazomoka in local terminology in Madagascar. Within the context of traditional practice, malaria (and/or malaria symptoms) is commonly treated by decoctions or infusions from bitter plants. One possible approach to the identification of new antimalarial drug candidates is to search for compounds that cure or prevent malaria in plants empirically used to treat malaria. Thus, it is worth documenting the ethnobotanical data, and testing the antiplasmodial activity of the extractive from plants.

Methods: We interviewed traditional healers, known locally as ombiasy, at Andasibe in the eastern, rainy part of Madagascar. We recorded details of the preparation and use of plants for medicinal purposes. We extracted five alkaloids from *Z. tsihanimposa* stem bark, and tested them in vitro against *Plasmodium falciparum* FCM29.

Results: We found that traditional healers treat malaria with herbal remedies consisting of one to eight different plants. We identified and listed the medicinal plants commonly used to treat malaria. The plants used included a large number of species from different families. *Zanthoxylum sp* (Rutaceae) was frequently cited, and plants from this genus are also used to treat malaria in other parts of Madagascar. From the plant list, *Zanthoxylum tsihanimposa*, bitter plant endemic to Madagascar, was selected and examined. Five alkaloids were isolates from the stem bark of this plant, and tested in vitro against malaria parasite. The geometric mean IC50 values ranged from 98.4 to 332.1 micromolar. The quinoline alkaloid gamma-fagarine exhibited the strongest antiplasmodial activity.

Conclusions: The current use of plants for medicinal purposes reflects the attachment of the Malagasy people to their culture, and also a lack of access to modern medicine. The possible extrapolation of these in vitro findings, obtained with plant extracts, to the treatment of malaria and/or the signs evoking malaria is still unclear. If plants are to be used as sources of novel antimalarial compounds, we need to increase our knowledge of their empirical use to improve plant selection. In the hope of preserving useful resources, we should now gather and record ethnobotanical data in Madagascar, and should try to bridge the gaps between empirics and realism.

Background

Malaria, which was initially recognized as episodic fever, was probably introduced into Madagascar from mainland Africa by immigration. Malaria was reported as early as 1602 on the coast of Madagascar [1]. This disease remains a public health problem [2]. The Malagasy words *tazo* and *tazomoka* refer to the clinical signs suggestive of malaria: principally fever, headache, backache, shivering and fatigue. Malagasy people, particularly those living in the countryside, use traditional plants to fight infectious diseases. The current use of plants for medicinal purposes reflects the attachment of the people to their culture and a lack of access to modern medicine (associated with poverty in most cases).

Local people are confident that they can recognize the signs of malaria http://www.pasteur.mg/AtlasPalu/pdspalu.htm. Within the context of traditional practice, malaria and/or malaria symptoms are treated by decoctions or infusions from bitter plants. Examined bitter plants reported to have traditionally antimalarial properties usually contain bitter compounds such as alkaloids, limonoids or quassinoids [3–7].

The resistance of *Plasmodium* spp. to drugs – such as chloroquine – has become a serious problem in areas of endemic malaria and in malaria-free areas with occasional imported cases. One possible approach to the identification of new antimalarial drug candidates is to search for compounds that cure or prevent malaria in plants empirically used to treat malaria. There is also a need to generate reliable scientific data to determine whether the plants currently used to treat malaria are actually effective. In the long term, this should help to prevent deaths due to ignorance and the misuse of plants for self-medication in the absence of advice from a qualified medical professional.

Materials and Methods

Ethnobotanical survey

We considered the traditional healers, known locally as *ombiasy*, to be the best source of information for our study. Field work was carried out in August 1993 in and around Andasibe (~130 km east of Antananarivo, the capital of Madagascar; 1,000 m above sea level). A retired forester introduced us to three local healers, aged between 53 and 89 years, all of good reputation in the area. Healers gave their consent. Drawing from the experience of Hammer and Johns [8], we interviewed these healers by means of informal, unstructured conversations. We thought that open discussion, rather than a rigid questionnaire, would provide the opportunity for healers to talk about their specialties and experiences and to provide as much detail as they wished. This loose structure also eliminated the pressure to provide answers to rigid questions, which might have lead to artificial responses, given purely to satisfy the researcher.

We obtained from these healers information relating to the identification of plants, their medicinal uses and the preparation of remedies. We focused particularly on the information provided concerning the plants used to treat *tazo* (malaria). There was considerable overlap between the information obtained from the three healers concerning the plants and treatments used. We, therefore, simply generated a combined pharmacopea for the three healers. The forester we worked with identified the plants from the given vernacular names. Samples were taken for all the plants listed, which were then compared with the type specimens from the National Park Herbarium, to ensure accurate identification.

The "principal" plants used as antimalarial which were cited independently by at least two of the three healers interviewed were listed by family and in alphabetical order (see plant list). We give both the scientific name of plant and the vernacular name, in that order. The list of "secondary" plants which may be mixed with the "principal" one is also added.

Chemical analysis and in vitro *antimalarial testing*

In targeting plants which might contain antimalarial compounds, attention was focused on the plants used to make infusions and decoctions to treat malaria. *Zanthoxylum tsihanimposa* (figures 1; 2 and 3) is known as a plant used in the treatment of malaria. It can be used alone (see plant list). The genus *Zanthoxylum* is used not only in the Andasibe region (eastern part of Madagascar), but also in the western parts of Madagascar (Rabarison & Randrianarivelojosia, *unpublished*). We, therefore, decided to collect and investigate *Z. tsihanim*posa. Plant material was collected from Ankarafantsika, in the wetter north-western part of Madagascar. The chemical extraction and analysis were carried out at the University of Natal, Durban, South Africa in 2001. The crude hexane extract of the stem bark of *Z. tsihanimposa* was fractionated by liquid chromatography over silica gel (Merck 9385) using a mixture of various proportions of dichloromethane and methanol. Antimalarial *in vitro* tests were performed at the Institut Pasteur de Madagascar. *In vitro* chemosensitivity tests were performed with the isotopic microtest method [9,10]. *P. falciparum* FCM29 strain resistant to chloroquine but susceptible to quinine – cultured in our laboratory according to the method described by Trager and Jensen [11] – was used to assess the antimalarial activity of the compounds tested. Quinine base (Sigma, St Louis, MO, USA) was tested as the reference antimalarial drug (figure 5). The antimalarial activity of the compound tested was expressed as the IC50 i.e. 50% inhibitory concentration [10].

Figure 1
Zanthoxylum tsihanimposa in Ankarafantsika forest (Photo: Milijaona Randrianarivelojosia)

Results and Discussion

Plants with antimalarial properties according to the traditional healers in Andasibe

AGAVACEAE
Dracaena reflexa Lamk. "hasina".

The leaf and bark decoction can be mixed with a selection of plants, including *Ficus megapoda, Nymphaea lotus, Noronhia linocerioides, Vepris ampody, Zanthoxylum madagascariense,* and *Gambeya boiviniana,* and is drunk (1 bowl, 3–4 times daily) to relieve malarial symptoms, tiredness, muscular aches and pains and poisoning.

Uses reported elsewhere: The branch leaves are used as a diuretic, the stem leaves to treat dysentery and diarrhea, and the aerial parts of the plant to treat dysmenorrhea [12]. It is also used to reduce fever and as a hemostatic treatment [12–14]. A decoction of the aerial parts of the plant is drunk as a febrifuge [15].

CANELLACEAE
Cinnamosma fragrans H. Bn. "sakarivohazo".

A decoction of the leaf and bark is drunk (1 bowl, 3–4 times daily) to relieve malarial symptoms, tiredness and muscular aches.

Uses reported elsewhere: The aerial parts are used to treat biliousness and as a diuretic [12]. The bark is used to treat stomach-aches and syphilis [12,13].

GRAMINEAE
Andropogon schœnanthus/nardis L. "veromanitra".

The leaf decoction is drunk with plenty of sugar and the fumes are inhaled as a febrifuge.

LEGUMINOSAE
Desmodium mauritianum D.C. de Candolle &*Desmodium hirtum* Grill and Perr. "tsilavindrivotro".

A decoction of the leaf and bark is mixed with a selection of five plants from the following list: *Ficus megapoda, Nymphaea lotus, Noronhia linocerioides, Vepris ampody, Zanthoxylum madagascariense, Gambeya boiviniana, Peddia involucrata.* This decoction is drunk (1 bowl, 3–4 times daily) to relieve malarial symptoms and tiredness.

Uses reported elsewhere: A decoction of the aerial parts of *Desmodium hirtum* is drunk to treat splenomegaly [15].

MALPIGHIACEAE
Tristellateia madagascariensis Poir "menahelika".

The leaf decoction is drunk (2–3 glasses a day) to treat malaria. Inhalation of steam from the decoction is used as a febrifuge.

MORACEAE
Ficus megapoda Bak. "mandresy".

A decoction of the leaf and bark is drunk (1 bowl, 3–4 times daily) to relieve malarial symptoms.

Uses reported elsewhere: A decoction of the leaf and root is drunk as a febrifuge [14,15].

NYMPHAEACEAE
Nymphaea lotus L. "voahirana" or "retsimilana".

Figure 2
Authentic voucher specimen of *Zanthoxylum tsihanimposa* fruits, kept at the Botany Department of the National Park, which we used for plant sample identification (Photo: Harison Rabarison)

Figure 3
Authentic voucher specimen of *Zanthoxylum tsihanimposa* leaves, kept at the Botany Department of the National Park, which used for plant identification (Photo: Harison Rabarison)

A decoction of the leaf and bark is mixed with a selection of plants from the following list: *Noronhia linocerioides*, *Vepris ampody*, *Zanthoxylum tsihanimposa*, *Peddia involucrata*. This mixture is drunk (1 bowl, 3–4 times daily) to relieve malarial symptoms, tiredness, muscular aches and pains, and poisoning.

Uses recorded elsewhere: Against malarial symptoms [14].

RUTACEAE

Vepris ampody H. Perr. "ampody"

A decoction of the leaf and bark is mixed with a selection of plants from the following list: *Cinnamosma fragrans*, *Desmodium mauritianum*, *Ficus megapoda*, *Zanthoxylum tsihanimposa*, *Gambeya boiviniana*, *Peddia involucrata*. This mixture is drunk (1 bowl, 3–4 times daily) to relieve malarial symptoms, tiredness, muscular aches and pains.

Zanthoxylum tsihanimposa Bak. "tsihanihimposa".

A decoction of the leaf and bark is used alone or mixed with a selection of plants from the following list: *Vepris ampody*, *Dracaena reflexa*, *Gambeya boiviniana*, *Peddia involucrata*. The decoction is drunk (1 bowl, 3–4 times daily) to relieve malarial symptoms, tiredness, muscular aches and pains, and poisoning.

THYMELAEACEAE

Peddiea involucrata Bak. "Montana".

A decoction of the leaf and bark is mixed with a selection of plants from *Vepris ampody*, *Zanthoxylum tsihanimposa*, and *Dracaena reflexa* and is drunk (1 bowl, 3–4 times daily) to relieve malarial symptoms, tiredness, muscular aches and pains, and poisoning.

The healers cited 58 plants from 33 families as being of medicinal value: 21% were used to treat *tazo* (malaria), 21% to cure skin problems (bleeding, rash, ulceration, wounds). The remaining plants were used for various purposes including the treatment of fatigue, headache, stomach-ache, diarrhoea, respiratory disorders and to drive bad spirits away from the house after a funeral.

Various plant preparations were used for treatment: decoction (51%) and infusion (24%) were the most common, followed by steaming and inhalation (11%), poultice/paste (7%), chewing of the plant material to release its juice (5%) and use of latex drops (1%). Leaf and stem bark were the most frequently used (62%). Roots (8%), stem (10%) and whole plant (9%) were also prescribed. Plants were generally used either fresh or dried and were prepared by traditional methods.

Belief and traditional practice

The strong spiritual beliefs of the rural communities of Madagascar are of particular relevance to the collection, preparation and administration of medicinal plants. The beliefs of the Malagasy people encompass not only the Christian god, but also ancestor spirits. All plants belong to God and it is, therefore, necessary to ask God's permission to use plants by means of prayers. The Malagasy believe that there is a balance between the harmful and curative powers of medicinal plants, and they pray to ancestral spirits to swing this balance towards healing and away from harm. The Malagasy people pray to the east, because they believe that there is a connection between God and the sun, both during and after collection, preparation and administration. Healers place salt at the base of the first plant of a set to be collected, because the order of collection is important. The time of day and the time of year are also relevant. It is thought best to collect material at full moon, because there is believed to be a connection between the phase of the moon and plant biology. Some plants are also preferentially collected at the beginning of the wet season, which lasts from September to April. Our survey suggested that healers classified plants as "principal" plants (which may be used alone) and "secondary" plants (which must be mixed with a principal plant). Cure duration varies from three to seven days. It was often mentioned that remedy should be given until recovery without any well defined duration. Such practice might increase toxic risk/hazard. Our survey in Andasibe showed that the *ombiasy* (healer, drug maker) combine the functions of diviner, traditional healer, and astrologer. Even highly educated person would not think of building a house without consulting the healer to determine the best day on which to begin the work. The survey demonstrated that traditional practice and traditional medicine are deeply rooted in Malagasy culture. Those who know about medicinal plants sometimes diagnose or treat themselves without first consulting a qualified medical practitioner or healer.

Secrecy

A healer's practices involve exploration, exploitation and secrecy. Before they will tell someone what they really use to treat sick people, they need to feel secure and that the person concerned is sincere. A healer told us that "*during the colonization period, when foreigners came to the rural village with Madagascan civil servants to ask about the use of plants to cure illness, some local people told them lies, indicating plants around their houses to which they attributed imaginary, nonsense uses*".

Suggested approach to target plant which might contain antimalarial compounds

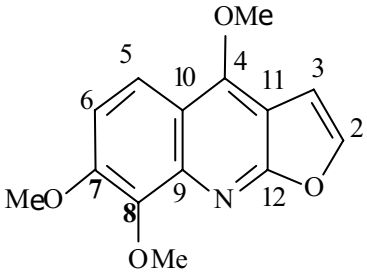
Individual plants are rarely used alone. In most cases, they are used as mixtures (see plant list). It will never be easy

to determine which plants are likely to be the most useful and should be examined to isolate pure active compounds. In traditional practice, *tazo* (malaria) is used as a general synonym for fever, shivering, muscle and joint pain, fatigue and headache. Some antimalarial plants are used for preparing baths or for inhalations (aromatic plants). It might, therefore, be useful to test the antibacterial, antiviral and anti-inflammatory activities of these groups of plants.

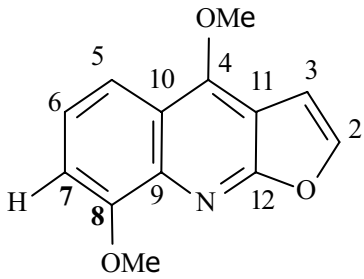
In targeting plants which might contain antimalarial compounds, attention should be focused on the plants traditionally used to make infusions and decoctions to treat *tazo*. Thus, we assessed the antiplasmodial activity of five alkaloids extracted from *Zanthoxylum tsihanimposa* stem bark. Our choice was based on the combination of the enthobotanical and the taxonomical approach in aim to target alkaloids at this stage of the study.

Antiplasmodial activity of alkaloids from Zanthoxylum tsihanimposa *against* P. falciparum *FCM29*

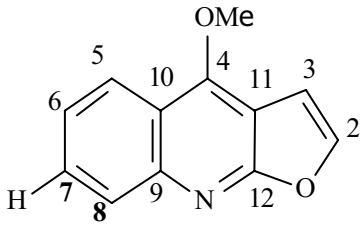
Purified compounds were identified by the use of nuclear magnetic resonance spectroscopy and mass spectrometric techniques. Five alkaloids – γ-fagarine, N-benzoyltyramine, skimmianine, dictamnine and 4-methoxy-1-2(1H)quinolinone – were isolated from the stem bark of *Z. tsihanimposa* (figure 4).



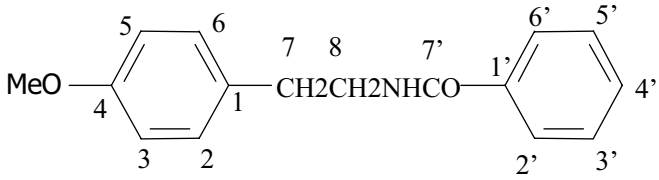
(**1a**) : skimmianine



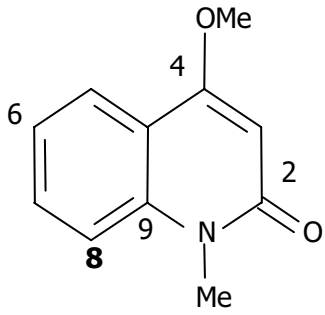
(**1b**) : γ-fagarine



(**1c**) dictamnine



(**1d**) : N-benzoyltyramine méthyl éther



(**1e**) : 4-methoxy-1-methyl-2(*1H*)-quinolinone

Figure 4
Chemical structure of alkaloids from *Zanthoxylum tsihanimposa* stem bark

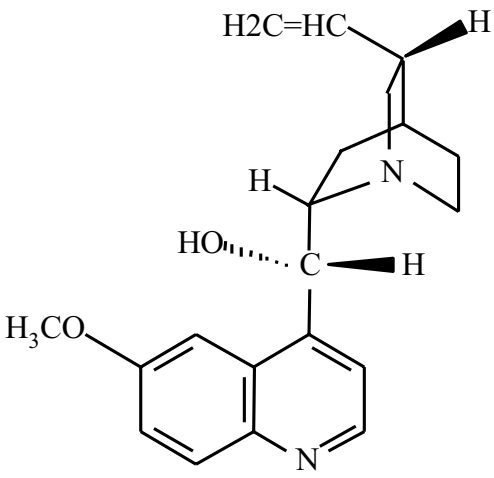


Figure 5
Chemical structure of quinine

The quinine IC50 geometric mean was 0.50 μM (95% CI: 0.48 – 0.52 μM). The geometric mean IC50 was 332.1 μM for dictamnine (95% CI: 205.1 – 459.1 μM), 270.7 μM for 4-methoxy-1-methyl-2(1H)-quinolinone (95% CI: 234.9 – 306.5 μM), 165.4 μM for N-benzoyltyramine methyl ether (95% CI: 89.1 – 241.3 μM), 134.3 μM for skimmianine (95% CI: 113.1 – 155.5 μM) and 98.4 μM for γ-fagarine (95% CI: 87.7 – 110.9 μM). These alkaloids exhibit antiplasmodial activity in the micromolar range. The quinoline alkaloid γ-fagarine was the most potent of the five alkaloids against *P. falciparum* FCM29 ($P < 0.05$). γ-fagarine was 3.4 times more active than dictamnine and and 1.4 times more active than skimmianine. The presence of the methoxy group in positions C4 and C8 seems to be important for bioactivity. However, these findings do not provide sufficient evidence to allow us to conclude that these alkaloids are the molecules primarily responsible for anti-*Plasmodium* activity of this plant. We will focus future efforts on the isolation of polar compounds from *Z. tsihanimposa* and on animal models for the testing of molecules *in vivo*. It is unclear how to extrapolate these *in vitro* findings obtained with plant extracts to the treatment of clinical malaria and signs of malaria. However, *in vitro* antimalarial testing makes possible the assessment of any compound intrinsic antiplasmodial activity. At this stage, it is unclear whether γ-fagarine, the most potent of the *Z. tsihanimposa* alkaloids tested, is truly effective against malaria. The IC50 value of γ-fagarine, in the micromolar range, like those of some antibiotics with antimalarial activity, is encouraging.

Concluding remarks

Knowledge about traditional medicinal practices and plants is currently transmitted from generation to generation principally by word of mouth. There is, therefore, little written information available about traditional Malagasy herbal medicine. Some of the existing documents – compiled in some cases by other authors – were prepared during the colonization period [12,13,16–21]. However, as mention above, given that some local people were known to have given false information in these surveys, it is difficult to determine which of the plants listed were really used as medicinal plants.

A remarkable 80% of the Malagasy flora is endemic to the island and found nowhere else. However, there is a risk that useful species will be lost due to the alarming rate of deforestation. Thus, we must now preserve the flora, hold more discussions with traditional practitioners about herbal medicine, document ethnobotanical information and evaluate scientifically ethnomedicinally used plants to bridge the gap between empirical treatment and realism. As antimalarial plants are usually prescribed to treat the symptoms suggestive of malaria, it is worthwhile testing crude extractives from plants such as *Z. tsihanimposa*, and also compounds isolated on the basis of bio-guided fractionation for antimalarial, antipyretic and anti-inflammatory activity and carrying out toxicity tests. Above all, observational studies on patients using antimalarial plants by use of guidelines produced by Research Initiative on Traditional Antimalarial Methods [Willcox M, *personal communication at the 3rd MIM Pan-African Malaria Conference*] needs to be done to get reliable information on the effects of traditional antimalarial treatments on human beings.

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