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Reference:

Sahar Traoré Mohamed, Aliou Baldé Mamdou, Camara Aïssata, Cos Paul, Maes Louis, Pieters Luc.- The malaria co-infection challenge : an investigation into the antimicrobial activity of selected Guinean medicinal plants
Journal of ethnopharmacology - ISSN 0378-8741 - 174(2015), p. 576-581
Full text (Publishers DOI): <http://dx.doi.org/doi:10.1016/j.jep.2015.03.008>

The malaria co-infection challenge: an investigation into the antimicrobial activity of selected Guinean medicinal plants

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Abstract

Ethnopharmacological relevance. In sub-Saharan Africa, concomitant occurrence of malaria and invasive infections with micro-organisms such as Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli* and yeasts or fungi such as *Candida albicans* and *Aspergillus fumigatus* is common. Non-tuberculous mycobacteriosis caused by *Mycobacterium chelonae* has been recognized as a pulmonary pathogen with increasing frequency without effective therapy. Although less important, the high incidence of *Trichophyton rubrum* infections along with its ability to evade host defense mechanisms, accounts for the high prevalence of infections with this dermatophyte. Considering the treatment cost of both malaria and microbial infections, along with the level of poverty, most affected African countries are unable to cope with the burden of these diseases. In sub-Saharan Africa, many plant species are widely used in the treatment of these diseases which are traditionally diagnosed through the common symptom of fever. Therefore it is of interest to evaluate the antimicrobial activities of medicinal plants reported for their use against malaria/fever.

Materials and Methods. Based on an ethnobotanical survey, 34 Guinean plant species widely used in the traditional treatment of fever and/or malaria have been collected and evaluated for their antimicrobial activities. Plants extracts were tested against *Candida albicans*, *Trichophyton rubrum*, *Aspergillus fumigatus*, *Mycobacterium chelonae*, *Staphylococcus aureus* and *Escherichia coli*.

Results. The most interesting activities against *Candida albicans* were obtained for the polar extracts of *Pseudospondias microcarpa* and *Ximenia americana* with IC₅₀ values of 6.99 and 8.12 µg/ml, respectively. The most pronounced activity against *Trichophyton rubrum* was obtained for the ethanol extract of *Terminalia macroptera* (IC₅₀ 5.59 µg/ml). Only 7 of the 51 tested extracts were active against *Staphylococcus aureus*. From these, the methanolic extracts of the leaves and stem bark of *Alchornea cordifolia* were the most active with IC₅₀ values of 2.81 and 7.47 µg/ml, respectively. Only *Terminalia albida* and *Lawsonia inermis* showed activity against *Mycobacterium chelonae*. None of the tested extracts was active against *Escherichia coli*.

Conclusion. A number of traditional Guinean plant species used against malaria/fever showed, in addition to their antiparasmodial properties, antimicrobial activity. The fact that some plant species are involved in the traditional treatment of malaria/fever without any antiparasmodial evidence may be justified by their antimicrobial activities.

Keywords: Antimicrobial; malaria; Co-infection; Guinea; plant extracts.

1. Introduction

Areas of the world with high rates of malaria also carry a heavy burden of infectious diseases which are caused by pathogenic microorganisms, such as parasites, bacteria, viruses or fungi. Within the poorest and developing countries in Africa, malaria, acute respiratory infections, diarrhea, tuberculosis and recently Ebola figure among the major infectious killers. Although having serious consequences through a prolongation of illness along with an increasing mortality especially to the vulnerable pregnant women and children infected with *Plasmodium falciparum*, the magnitude and the impact of malaria co-infection with other pathogenic microorganisms are still largely unknown. Until now, it was suggested that the most important cause of death among children in Africa is malaria; however, the methodology of these studies has been questioned. More recent community-based studies of the incidence of invasive bacterial infections in rural Gambia and Kenya have all documented a significant contribution to childhood morbidity and mortality in developing countries. One of the risk factors to develop invasive bacterial infections in Africa is *Plasmodium falciparum* malaria [Anthony *et al.*, 2009; Scott *et al.*, 2011; Bassat *et al.*, 2009; Bronzan *et al.*, 2007; Church *et al.*, 2014].

In sub-Saharan Africa, concomitant occurrence of malaria and invasive infections by microorganisms is common. In children with severe *Plasmodium falciparum* malaria, evidence of invasive bacterial infections with Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli*, and other enteric Gram-negative bacteria has been reported in many countries including Tanzania, Kenya, Mozambique, Nigeria and Burkina Faso [Berkley *et al.*, 1999; 2005; Brent *et al.*, 2006; Chaturvedi *et al.*, 2009; Church *et al.*, 2014; Crawley *et al.*, 2010; Evans *et al.*, 2004; Graham *et al.*, 2000; Gwer *et al.*, 2007; Keong *et al.*, 2006; Maltha *et al.*, 2014; Uneke *et al.*, 2008; Walsh *et al.*, 2000]. Gram-negative bacteria like *E.coli* may cause amongst others urinary tract infections, pneumonia, neonatal meningitis, diarrhea and skin infections; while Gram-positive organisms like *S. aureus* may cause nosocomial infections, skin infections, respiratory diseases, meningitis, endocarditis, osteomyelitis and wound infections [Gunaselvi and Kulasekaren, 2010].

The major yeasts and fungi implicated worldwide as a potential cause of invasive fungal infections include *Candida* and *Aspergillus* spp. These produce a wide variety of infections that are difficult to diagnose as most of the diagnostic tests are non-specific and the culture takes a long time. *C. albicans* can cause infections in specific physiological and pathological conditions such as infancy, pregnancy, diabetes, prolonged broad spectrum antibiotic

administration, steroidal chemotherapy as well as AIDS [Low and Rotstein, 2011]. Aspergillosis is one of many opportunistic fungal infections that mainly affect the lungs [Silva, 2010] and 90% of invasive aspergillosis is caused by the air-borne opportunistic fungal pathogen, *Aspergillus fumigatus*. The mortality rate of this disease is still very high (50-95%), partly because of diagnostic difficulties, limited antifungal treatment options, and the weak condition of patients at risk. But also in part because understanding of virulence factors involved in *A. fumigatus* pathogenicity and interactions of the pathogen with the host immune system is still poor [Binder and Lass-Flörl, 2013; McCormick *et al.*, 2010].

Dermatophytic fungal infections are one of the most common infectious diseases and are among the most commonly diagnosed skin diseases in Africa [Nweze, 2010]. Although the correlation between malaria and these fungal infections is not documented, they are present worldwide. *Trichophyton rubrum* is responsible for the vast majority of chronic dermatophytoses [Scheers *et al.*, 2013]. Its high infectivity and its ubiquitous presence account for its high incidence. Together with the ability of *T. rubrum* to evade host defense mechanisms, this accounts for the high prevalence of infections with this fungus [Dahl and Grando, 1994]. Their co-infection with malaria must be common but is poorly documented particularly in malaria endemic areas.

Non-tuberculous mycobacteriosis with *Mycobacterium chelonae* is an opportunistic pathogen which has been recognized as a pulmonary pathogen with increasing frequency. It is an increasingly recognized cause of disease in immunocompromised patients. *M. chelonae* is characterised by a high degree of *in vitro* resistance to antituberculous drugs and has been associated with development of drug resistance and treatment failures. Attempts to eradicate the organism through chemotherapy have been largely unsuccessful. No effective therapy for *M. chelonae* lung infections has been established to date, and reported cases of pulmonary resection for the treatment of *M. chelonae* infections are extremely rare [Singh and Yu, 1992; Green *et al.*, 2000; Goto *et al.*, 2012; Wallace *et al.*, 2001].

Since routine antibiotics along with antimalarials are currently recommended for patients with severe malaria, the indiscriminate use of antibiotics would be both financially costly and could perpetuate the rise of antimicrobial resistance, which threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi [WHO, 2014]. Considering the treatment cost of both malaria and microbial infections, along with the level of poverty, most affected African countries are unable to cope with the burden of these diseases. For many African people, particularly the rural populations,

traditional medicines continue to be the first and most important source of medical solace when illness strikes health. Thus, many plant species are widely used in the treatment of various diseases as a recipe consisting of only one or more medicinal plants. Moreover, the same plant species or recipe could be frequently and indistinctly employed for the traditional symptomatic treatment of various diseases such as malaria, bacterial and viral infections. In Guinean rural areas where diagnosis based on blood cultures is usually unavailable and antibiotic choice is limited, traditional medicine is the unique way for the management of most of the diseases. Owing to the fact that it is very difficult for traditional healers to differentiate between malaria and other infectious diseases, their remedies mainly aim to treat the fever symptom.

From an ethnobotanical survey on malaria/fever conducted in Guinea, numerous plant species have been collected [Traoré *et al.*, 2013], but only few of them exhibited an *in vitro* antimalarial potency with an $IC_{50} < 64 \mu\text{g/ml}$. To justify the “antimalarial” traditional use of the weakly active or inactive plant species against *Plasmodium falciparum* ($IC_{50} \geq 64 \mu\text{g/ml}$), it was assumed that these could possibly act on symptoms of malaria such as febrile illnesses and/or enhance immunological responses [Traoré *et al.*, 2014]. Upon these considerations, it is of interest to clarify the biological importance and level of antimicrobial and/or antimalarial activity of the plant species cited by the Guinean traditional healers in the treatment of fever/malaria. Nowadays, a worldwide search for new classes of effective antimalarial and antibacterial drugs is in progress and natural products have been recognized as highly important candidates for such purpose [Tobinaga *et al.*, 2009]. Therefore the present study was undertaken.

2. Materials and Methods

2.1. *Ethnobotanical investigation*

The selected plants were collected during an ethnobotanical survey conducted in the four main Guinean regions from May 2008 to September 2010. Botanical identification was first conducted in the field, and confirmed by Dr. S.M. Keita (CERE, University of Conakry), M.S. Barry and N. Camara (Centre de Recherche et de Valorisation des Plantes Médicinales – CRVPM, Dubreka). Voucher specimen registration numbers at the Herbarium of the CRVPM and local names are listed in Traoré *et al.*, 2013. Traditional healers were interviewed in their homes, and herbalists in front of their stalls (on the roadside or on various market places). The questionnaire and oral interviews were based on the standardised model which was designed by CRVPM, Dubreka. The main questions focused on demographic data (age, sex),

educational level, professional experience, knowledge about malaria: local names, cause, known signs and symptoms of malaria, plants used in the preparation of antimalarial remedies, plant parts employed, mode of preparation, and mode of administration.

2.2. Preparation of plant extracts

Plant extracts were prepared by macerating 10 g of powdered dried plant material with 50 ml solvent (hexane, chloroform, methanol, or ethanol 70%) for 24 h. With regard to the aqueous crude extract, a decoction of 10 g dried plant powder was prepared in 150 ml distilled water for 30 min, corresponding to the traditional preparation method. The extracts were then filtered and each filtrate was evaporated under reduced pressure to dryness; 5 mg were weighed and submitted for antimicrobial testing.

2.3. Biological evaluation

Antimicrobial evaluation was carried out as previously described by Cos *et al.* [2006a; 2006b]. Extracts were tested on the following microorganisms: the enterobacteriaceae Gram-negative *Escherichia coli* ATCC 8739, the Gram-positive cocci *Staphylococcus aureus* ATCC 6538, the acid-fast bacteria *Mycobacterium chelonae*, the yeast *Candida albicans* ATCC 59630, the opportunistic filamentous fungi *Aspergillus fumigatus* ATCC 16404, the dermatophyte *Trichophyton rubrum* ATCC 68183. The level of antimicrobial activity was arbitrarily ranked according to the following criteria: strong ($IC_{50} \leq 10 \mu\text{g/ml}$); good ($10 \mu\text{g/ml} < IC_{50} \leq 20 \mu\text{g/ml}$); moderate ($20 \mu\text{g/ml} < IC_{50} \leq 40 \mu\text{g/ml}$); weak ($40 \mu\text{g/ml} < IC_{50} \leq 64 \mu\text{g/ml}$); inactive ($IC_{50} \geq 64 \mu\text{g/ml}$). Positive control substances included flucytosine for *Candida albicans* (IC_{50} 0.34 μM), voriconazole for *Trichophyton rubrum* and *Aspergillus fumigatus* (IC_{50} 0.18 μM and 0.75 μM , respectively), doxycycline for *Staphylococcus aureus* and *Escherichia coli* (IC_{50} 0.83 μM and 0.82 μM , respectively) and rifampicin for *Mycobacterium chelonae* (IC_{50} 0.1 μM).

3. Results and Discussion

Since the clinical features of malaria closely resemble those of other febrile illnesses, such as typhoid fever, septicemias, urinary tract infections, upper and lower respiratory tract infections, which are common in most of the tropical countries, antimalarial plant species with antimicrobial properties are of importance. The plants species were selected on the basis of their number of citations by traditional healers for their use against malaria which in fact include febrile illnesses. Of the 51 extracts from 34 plant species tested, only 17 (from 15 plant species) showed strong to weak antimicrobial activity ($IC_{50} < 64\mu\text{g/ml}$). The 34 selected plant species are distributed into the following 24 families: Anacardiaceae (*Pseudospondias microcarpa* (A.Rich.) Engl.; *Spondias mombin* L.), Annonaceae (*Cleistopholis patens* Engl. & Diels), Apocynaceae (*Strophanthus hispidus* DC), Bignoniaceae (*Newbouldia laevis* (P.Beauv.) Seem.; *Markhamia tomentosa* (Benth.) K.Schum), Caesalpiniaceae (*Cassia sieberiana* DC; *Mezoneuron benthamianum* Baill.; *Piliostigma thonningii* (Schumach) Milne-Redhead), Caricaceae (*Carica papaya* L.), Combretaceae (*Combretum glutinosum* Perr., *Guiera senegalensis* J.F.Gmel., *Terminalia albida* Sc. Elliot, *Terminalia macroptera* Guill.); Dilleniaceae (*Tetracera alnifolia* Willd.), Ebenaceae (*Diospyros mespiliformis* Hochst.), Euphorbiaceae (*Alchornea cordifolia* (Schumach.&Thonn.) Müll.Arg., *Bridelia micrantha* (Hochst.) Baill), Fabaceae (*Erythrina senegalensis* DC), Hymenocardiaceae (*Hymenocardia acida* Tul.), Hypericaceae (*Vismia guineensis* (L.) Choisy), Icacinaceae (*Rhaphiostylis beninensis* (Hook f.) Planch.), Lythraceae (*Lawsonia inermis* L.), Meliaceae (*Azadirachta indica* A. Juss.; *Trichilia emetica* Vahl.), Mimosaceae (*Albizia zygia* (DC.) J. F. Macbr), Moraceae (*Ficus sp*; *Ficus vallis-choudae* Del.), Olacaceae (*Ximenia americana* L.), Rubiaceae (*Morinda geminata* DC), Rutaceae (*Zanthoxylum zanthoxyloides* (Lam.) Zepernick& Timber), Sapindaceae (*Paullinia pinnata* L.), Tiliaceae (*Grewia villosa* Willd.), Verbenaceae (*Lantana camara* L.)

A total of 51 extracts from 34 plant species were evaluated for their antibacterial and antifungal activity against *C. albicans* (34 extracts from 31 plant species), *T. rubrum* (26 extracts from 24 plant species), *A. fumigatus* (7 extracts from 7 plant species), *S. aureus* (51 extracts from 34 plant species), *E.coli* (47 extracts from 30 plant species) and *M. chelonae* (10 extracts from 9 plant species). The cytotoxicity of all the tested extracts has been established previously [Traoré *et al.*, 2014].

A total of 26, 17, 6 and 2 plant extracts were prepared from leaves, stem bark, bark, and root bark, respectively. Among these, 42 were polar (23 methanol, 14 ethanol 70%, 5 aqueous extracts), and 9 were apolar (6 hexane, 3 chloroform). The maximum concentration tested for each sample was 64 µg/ml. Since for all anti-effective bioassays of extracts tested against Gram-negative bacteria, mycobacteria and fungi, IC₅₀ values should be below 100 µg/ml to be considered as active [Cos *et al.*, 2006], all tested samples with an IC₅₀ > 64 µg/ml (*S. aureus*, *M. chelonae*, *C. albicans*, *T. rubrum* and *A. fumigatus*) could not be considered strictly speaking as inactive. But, based on arbitrarily defined criteria, any IC₅₀ value above 64 µg/ml was considered as inactive in the present study.

3.1. Antifungal activity:

Candida albicans

Except *Ficus* spp, *H. acida* and *S. mombin*, all the other 31 plant species were tested. This yeast was inhibited by only 6 plant extracts: *P. microcarpa* (IC₅₀ 6.99 µg/ml; methanol extract of stem bark) and *X. americana* (IC₅₀ 8.12 µg/ml; methanol extract of leaf), *D. mespiliformis* (IC₅₀ 21.69 µg/mL; ethanol extract of leaf), *C. glutinosum* (IC₅₀ 28.12 µg/mL; methanol extract of leaf), *T. macroptera* (IC₅₀ 21.93 µg/ml; ethanol extract of stem bark) and *T. albida* (IC₅₀ 34.55 µg/ml; methanol extract of root bark). The other 25 tested plant species were not active at a concentration ≤ 64 µg/ml.

C. albicans is known as a common cause of morbidity and mortality in immune-compromised individuals [Lee *et al.*, 2002]. Previous investigations indicated a good activity against *C. albicans* for the chloroform extract of the leaf of *X. Americana*, while the methanol and aqueous extracts were devoid of any activity [Omer and Elnima, 2003]. A weak activity was also described for the methanol and aqueous extracts of the stem bark [Maikai *et al.*, 2009]. On the other hand, our results with *P. microcarpa* contrasted with the inactivity reported by Kisangau *et al.* [2007]. All the active extracts against *C. albicans* are also active against *T. rubrum*, except *C. glutinosum* and *M. geminata*, which were only active against *C. albicans* and *T. rubrum*, respectively.

The antifungal properties of the tested plant species were in agreement with some previous results, such as the wide antifungal effect of the polar extracts of *X. americana* (leaf, stem-bark, or root) against *C. albicans*, *A. niger*, *Saccharomyces cerevisiae* [Omer and Elnima, 2003], the significant antifungal activity of *D. mespiliformis* against *A. flavus*, *A. niger*, *Microsporum gypseum*, *T. rubrum* and *C. albicans* [Sadiq *et al.*, 2013], the activity of *A.*

cordifolia (leaf, stem-bark) against *C. albicans* [Adeshina *et al.*, 2012]. The antifungal activities of *Terminalia* species are related to the presence of tannins and saponins [Baba-Moussa *et al.*, 1999]. However, our results on the antifungal activity of *T. macroptera* contrasted with its inactivity described by Silva *et al.* [1997].

Aspergillus fumigatus

At the highest tested concentration (64 µg/ml), none of the seven tested extracts from *Bridelia micrantha*, *Combretum glutinosum*, *Lantana camara*, *Pseudospondias microcarpa*, *Raphiostylis beninensis*, *Ximenia americana* and *Zanthoxylum zanthoxyloides* were active. Nevertheless, for related species such as *Bridelia atroviridis* and *Zanthoxylum gillettii*, antifungal activity against *Aspergillus niger* has been reported [Agyare *et al.*, 2006]. According to Saadabi (2006), the CHCl₃ leaf extract of *Grewia villosa* was strongly active against *Aspergillus flavus*, *Candida albicans* and slightly active against *A. fumigatus* and *A. niger*. These opportunistic filamentous fungi were considered for years to be weak pathogens. With an increasing number of immunosuppressed patients, however, there has been a dramatic increase in severe and usually fatal invasive aspergillosis, which now is the most common mold infection worldwide [Latgé, 1999].

Trichophyton rubrum

Only the ethanol extract of bark of *T. macroptera* (IC₅₀ 5.59 µg/ml), the ethanol extract of the root of *M. geminata* (IC₅₀ 19.5 µg/ml), the ethanol extract of leaf of *D. mespiliformis* (IC₅₀ 24.44 µg/ml), the methanol extract of leaf of *X. americana* (IC₅₀ 26.06 µg/ml) and the methanol extract of bark of *P. microcarpa* (IC₅₀ 28.15 µg/ml) showed a significant activity. The other 19 tested plant species were devoid of any activity at the highest concentration of 64 µg/ml (*A. zygia*, *A. indica*, *B. micrantha*, *C. sieberiana*, *C. patens*, *C. glutinosum*, *E. senegalensis*, *F. vallis-choudae*, *G. villosa*, *G. senegalensis*, *L. camara*, *M. tomentosa*, *P. pinnata*, *P. thonningii*, *R. beninensis*, *S. hispidus*, *T. emetic*, *V. guineensis*, and *Z. zanthoxyloides*).

3.2. Antibacterial activities

Escherichia coli

The Gram-negative *E. coli* was the least susceptible among the 3 tested micro-organisms; none of the 47 tested extracts were active at the highest tested concentration (64 µg/ml). However, previous activities against *E. coli* have been described for *A. cordifolia*, *Bridelia*

micrantha, *S. mombin*, *T. albida*, *L. inermis*, *L. camara*, *R. beninensis* [Adeyemi *et al.*, 2008; Corthout *et al.*, 1994; Gull *et al.*, 2013; Ayodole *et al.*, 2010; Ganjewala *et al.*, 2009; Barreto *et al.*, 2010; Lasisi *et al.*, 2011].

Staphylococcus aureus

All the extracts were tested against the Gram-positive *S. aureus* and 11/51 (21.56 %) showed activity with an IC₅₀ less than 64 µg/ml. The best antibacterial activity was observed for the methanolic extracts of the leaves and stem barks of *Alchornea cordifolia* (IC₅₀ values 2.18 and 7.47 µg/ml, respectively), the methanol and hexane extracts of the leaves of *S. mombin* (IC₅₀ 11.81, and 19.97 µg/ml, respectively), and the methanol extract of the leaves of *L. camara* (IC₅₀ 14.35 µg/ml). The hexane extract of the root bark of *V. guineensis*, the methanol extract of the leaves of *T. alnifolia* and the stem-bark of *Ficus* sp. exhibited a moderate effect against *S. aureus* (IC₅₀ 24.41, 26.91 and 29.23 µg/ml, respectively), while the extracts of *Guiera senegalensis* (ethanol leaf), *Hymenocardia acida* aqueous stem bark) and *Morinda geminata* (ethanol root bark) were weakly active (IC₅₀ 46.59, 58.69 and 61.17 µg/ml, respectively).

The antibacterial activity of some of these plant species has extensively been studied. Among these, *A. cordifolia* (leaf, stem-bark) showed a wide and significant inhibition of many pathogenic microorganisms such as *S. aureus* and *Pseudomonas aeruginosa* [Okeke *et al.*, 1999; Igbeneghu *et al.*, 2007], *Bacillus subtilis* and *Klebsiella pneumonia* [Ajali, 2000]. Some *A. cordifolia* fractions from the most active leaf extract, notably those containing phenolics and terpenoids, exhibited significant activity against *P. aeruginosa*, *B. subtilis* and *E. coli* [Ganjewala *et al.*, 2009]. *S. mombin* showed a moderate activity against, *S. aureus*, *K. pneumoniae*, *Salmonella typhosa*, *Serratia marcescens*, *Proteus mirabilis* and *Enterobacter cloacae* [Abo *et al.*, 1999; Corthout *et al.*, 1994; Umeh *et al.*, 2009; da Silva *et al.*, 2012]. *T. albida* and *L. inermis* exhibited an antimicrobial activity against *S. aureus*, *Klebsiella pneumoniae*, *E. coli*, *Streptococcus pyogenes*, *P. aeruginosa* and *P. mirabilis* [Gull *et al.*, 2013; Ayodole *et al.*, 2010]. The leaf extract of *Lantana camara* was found to be effective against *B. subtilis*, *P. aeruginosa*, *S. aureus*, *P. vulgaris* and *V. cholerae* [Ganjewala *et al.*, 2009; Barreto *et al.*, 2010].

Mycobacterium chelonae

The methanol extract of the root bark of *Terminalia albida* and the leaf of *Lawsonia inermis* were the only samples active against *M. chelonae* with interesting IC₅₀ values of 11.81 and 16 µg/ml, respectively. This result could be interesting as compared with some recent *in vitro*

susceptibility studies demonstrating activity of newly developed antimicrobials such as linezolid which inhibited *M. chelonae* with a MIC 50% at 8 µg/ml or MIC 90% at 16 µg/ml [Wallace *et al.*, 2001]. The antimycobacterial activity of the leaf extract of *L. inermis* (IC₅₀ 16 µg/ml) against non-tuberculous mycobacteriosis caused by *M. chelonae* overlapped with previous reported activity against *M. tuberculosis* H37Rv which was inhibited by 6 µg/ml of *L. inermis* extract. Moreover, in *in vivo* studies on guinea pigs and mice at a dose of 5 mg/kg body weight, the herb showed significant resolution of experimental tuberculosis following infection with *M. tuberculosis* H37Rv [Sharma, 1990]. Such activities could be related at least in part to the presence of lawsone, i.e. 2-hydroxynaphthoquinone, one of the major bioactive constituents of *L. inermis* [Lall *et al.*, 2003] and lawsonicin, which showed an IC₅₀ value of 6.25 µg/ml against *M. tuberculosis* H37Rv [Bhatti *et al.*, 2013]. Moreover, our current findings on the antimycobacterial properties of *T. albida* (IC₅₀ 11.81 µg/ml) are in line with earlier reports on *Terminalia* species particularly *T. avicennioides*, which inhibited significantly the growth of *M. tuberculosis* and *Bacillus Calmette Guerin* (BCG) at 78 and 200 µg/ml, respectively [Mann *et al.*, 2008]. Like other oleanane triterpenoids, friedelin isolated from *T. avicennioides* exhibited an *in vitro* antimycobacterial activity against BCG with a MIC value of 4.9 µg/ml [Abdullahi *et al.*, 2011]. On the other hand, the bark and root are used as an antibiotic in Nigeria [Ayodele *et al.*, 2010]. Noteworthy, the two *Terminalia* species *viz.* *T. albida* and *T. macroptera* figure among the most frequently cited antimalarial plant species by Guinean traditional healers and herbalists [Traoré *et al.*, 2013]. Although inactive against *M. chelonae* at the highest tested concentration (64 µg/ml), the leaf and root bark of *Tetracera alnifolia* were active against *M. tuberculosis* [Lawal *et al.*, 2011]. Neem seed oil and essential oils from leaves and bark have been shown to inhibit the growth of various genera of pathogenic microorganisms, such as *Mycobacterium* and *Plasmodium* [Habluetzel *et al.*, 2009].

Based on our previous *in vitro* antiplasmodial study of Guinean plant species [Traoré *et al.*, 2014], the relationship between the *in vitro* antimicrobial and antimalarial activities of the tested plant species is heterogeneous:

4/34 (12%) were devoid of any antimicrobial or antiplasmodial activity against *P. falciparum*-K1 at the highest tested concentration (IC₅₀ >64 µg/ml), *i.e.* *Bridelia* sp., *Raphiostylis beninensis*, *Strophantus hispidus* and *Zanthoxylum zanthoxyloides*;

3/34 (9%) were only antimicrobially active, viz. *Pseudospondias microcarpa* (*C. albicans*: IC₅₀ 6.99 µg/ml), *Tetracera alnifolia* (*S. aureus* IC₅₀ 26.91 µg/ml) and *Ximenia americana* (*T. rubrum* IC₅₀ 8.12 µg/ml);

14/34 (41%) were only antiplasmodially active [Traoré *et al.*, 2014]. The relative absence of antimicrobial activity of these plant species could favor of their nearly exclusive antimalarial use. The most important antiplasmodial effects against *P. falciparum*-K1 were observed for *Mezoneuron benthamianum* (IC₅₀ 5.8 µg/ml), *Newbouldia laevis* (IC₅₀ 9.1 µg/ml), *Albizia zygia* (IC₅₀ 18.1 µg/ml), *Carica papaya* (IC₅₀ 11.4 µg/ml), *Ficus vallis-choudae* (IC₅₀ 16.3 µg/ml), *Paullinia pinnata* (IC₅₀ 17.3 µg/ml) and *Azadirachta indica* (IC₅₀ 5.8 µg/ml) [Traoré *et al.*, 2014];

13/34 (38%) of the tested plant species showed antimicrobial as well as antiplasmodial [Traoré *et al.*, 2014] activities. Among these, strong to moderate inhibition was observed for the polar extracts of *A. cordifolia* (*S. aureus*: IC₅₀ 2.81 to 7.47 µg/ml; *P. falciparum*: IC₅₀ 9.3 – 11 µg/ml), the methanol extract of *S. mombin* (*S. aureus*: IC₅₀ = 11.81 µg/ml; *P. falciparum*: IC₅₀ 2.8 µg/ml), the chloroform extract of *V. guineensis* (*S. aureus*: IC₅₀ 24.41 µg/ml; *P. falciparum*: IC₅₀ 1.9 µg/ml), the methanol extract of *T. albida* (*C. albicans* and *M. chelonae*: IC₅₀ 34.55 and 11.81 µg/ml, respectively; *P. falciparum*: IC₅₀ 0.6 µg/ml), the aqueous ethanolic extract of *T. macroptera* (*C. albicans* and *T. rubrum*: IC₅₀ 21.93 and 5.59 µg/ml, respectively; *P. falciparum*: IC₅₀ 6.8 µg/ml), the methanol extract of *L. inermis* (*M. chelonae*: IC₅₀ 16 µg/ml; *P. falciparum*: IC₅₀ 23.4 µg/ml) and the methanol extract of *L. camara* (*S. aureus*: IC₅₀ 14.35 µg/ml; *P. falciparum* IC₅₀ 24.4 µg/ml).

Failure to treat concurrent bacterial infections in children with malaria may lead to severe morbidity and mortality. Taking into account that severely ill patients with complicated *P. falciparum* malaria are also profoundly immunosuppressed and susceptible to opportunistic fungal infections [Soesan *et al.*, 1993; Däbritz *et al.*, 2011], and that there is evidence of altered immune function in children with malaria [Okwara *et al.*, 2004], the antimalarial plant species with an evidence of antimicrobial properties are of interest. On the other hand, the fact that some plant species are involved in the treatment of diseases with febrile symptoms without any antiplasmodial evidence could be justified by their antimicrobial activities.

Patients with complicated *P. falciparum* malaria are also profoundly immunosuppressed and susceptible to opportunistic infections. The frequent presence of complicating pathologies in relatively non-immune young children with malaria contributes to a high morbidity and mortality. In addition the diagnosis of malaria in traditional medicine is tentative and mainly

based on fever symptoms, which is common to many other microbial infections as well. Although the present antimicrobial investigation was not exhaustive, these preliminary results may justify at least partly the traditional use of some “antimalarial” plant species without any *in vitro* or *in vivo* antiplasmodial evidence. To the best of our knowledge, this is the first report on the antimycobacterial activity of *T. albida* and *L. inermis* against *M. chelonae*. This finding could be of interest since *L. inermis* and *Terminalia* spp. have been reported to be active against *M. tuberculosis*. Moreover, the antiplasmodial activity previously described for *T. albida* (0.6 µg/ml) [Traoré *et al.*, 2014] may be of therapeutic importance in the treatment of malaria associated with cough in view of its favourable Selectivity Index (SI) >100). Further studies are required to validate and rationalise such considerations. Future perspectives include a wide antimicrobial testing of all other antimalarial plant species, the identification of active antimalarial and/or antimicrobial constituents, the evaluation of the toxicity of the most interesting plant species along with a clinical trial to evaluate the efficacy and tolerability of standardised plant extracts.

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