ELSEVIER

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology



journal homepage: www.elsevier.com/locate/jethpharm

Ethnopharmacological survey of Annonaceae medicinal plants used to treat malaria in four areas of Cameroon

Nolé Tsabang^a, Patrick Valère Tsouh Fokou^b, Lauve Rachel Yamthe Tchokouaha^b, Béatrice Noguem^a, Issakou Bakarnga-Via^b, Mireille Sylviane Dongmo Nguepi^b, Bernard Aloys Nkongmeneck^c, Fabrice Fekam Boyom^{b,*}

^a Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaoundé, Cameroon

^b Laboratory of Phytobiochemistry and Medicinal Plants Studies, Department of Biochemistry, University of Yaoundé I, P.O. Box 812 Yaoundé, Cameroon

^c Department of Plant Biology and Physiology, University of Yaoundé I, P.O. Box 812 Yaoundé, Cameroon

ARTICLE INFO

Article history: Received 1 June 2011 Received in revised form 26 October 2011 Accepted 28 October 2011 Available online 4 November 2011

Keywords: Antimalarial Annonaceae Ethnopharmacological survey Ethnopharmacological preparation Cameroon Traditional medicine

ABSTRACT

Ethnopharmacological relevance: Malaria endemic countries have vital resources that are medicinal plants on which their traditional medicines depend. In some Cameroonian settings, in addition to the commonly used potions from plants like *Alstonia boonei, Zanthoxylum macrophylla* and *Mangifera indica*, other herbal species are being increasingly used to treat malaria. So, specialized traditional healers have developed alternative reasonably priced therapies, relying on the signs and/or symptoms of malaria. Within this framework, Annonaceae plants were found to be increasingly utilized and therefore, highlighting the need to document this traditional knowledge for better malaria control.

Materials and methods: Interview approach was used to document indigenous knowledge, usage customs and practices of Annonaceae species in the treatment of malaria in four Cameroonian areas (Yaoundé and its surroundings, Ngoyang, Kon-Yambetta and Mbalmayo).

Results: A total of 19/30 users of plants accepted to share their experiences during a semi-structured survey. Twelve of the respondents were men and seven were women. Thirty recipes based on twenty-one plants were recorded.

Conclusion: Annickia chlorantha was the only plant commonly found in the four study sites. Seven species of Annonaceae were found to be used to treat malaria, while 14 were used to treat symptoms that might be related to malaria.

© 2011 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Malaria is the major pathology in the tropics. So despite the benefits of modern medicine, malaria remains a serious public health problem for one of the main reasons: anti-malarial drugs are generally expensive for people in endemic countries, in addition to drug resistant *Plasmodium falciparum*, which extends even to the latest drugs (Dondorp et al., 2009).

Overall, 3 billion people are at risk of malaria infection in 109 countries and territories, with infections causing disease in 300–500 million people and killing about 1 million people annually (Hay et al., 2010). Pregnant women and children less than five years are at the greatest risk of serious morbidity.

This disease is a major obstruction to socioeconomic development in endemic countries especially in Sub-Saharan Africa. It constitutes about 10% of Africa's total disease burden; 40% of its health expenditure and 30–50% of inpatient cases (World Malaria Report, 2010). Consequently, thousands of people continue to die from malaria each year and people living in endemic areas are all at risk.

Cameroon is amongst the top 18 countries where 90% of recorded deaths due to malaria occur (World Malaria Report, 2010). In daily practice, the huge majority of the Cameroonian people recognizes and utilizes the properties of several plants to combat malaria and other diseases (Tsabang, 2008). Amongst these, current trends indicate that the Annonaceae are increasingly used and have been proved to be promising source of antimalarial agents (Boyom et al., 2003, 2009, 2011a,b; Boyom, 2004).

The aim of the present study was to collect and document information on the traditional use of medicinal plants for the treatment of malaria by Bakola pygmies, Yambetta and cosmopolitan populations of Yaoundé and Mbalmayo in Cameroon. The outcome of interactions with information providers indicated Annonaceae as a promising plant family. Therefore, we have decided to report on this family. Specifically, the study sought to document the species

^{*} Corresponding author at: Department of Biochemistry, Faculty of Science, University of Yaoundé I, P.O. Box 812 Yaoundé, Cameroon. Tel.: +237 7727 6585. *E-mail address*: ffefe@yahoo.com (F.F. Boyom).

^{0378-8741/\$ –} see front matter @ 2011 Elsevier Ireland Ltd. All rights reserved. doi:10.1016/j.jep.2011.10.035



Fig. 1. Study sites.

of Annonaceae used, methods of preparation and administration of the herbal remedies by the traditional herbal practitioners to treat malaria. It is hoped that the results of this study will provide the basis for further pharmacological studies that are critical for the development of alternative antimalarial drugs.

2. Methodology

2.1. Sites of study and justification

In the framework of this study, four sites were chosen in the centre and South regions of Cameroon (Fig. 1): Yaoundé and its surroundings, Kon-Yambetta, Mbalmayo (Centre region) and Ngoyang (South). The following reasons have motivated the choice of these areas.

Yaoundé area and its surroundings (1): This is an area of semideciduous forest strongly degraded by the population burden. It is a cosmopolitan site where people from various areas and cultures cohabit. Environmental degradation and mismanagement foster the development of malaria vectors (mosquitoes), thereby fostering the level of parasite transmission. Due to the high level of malaria transmission in this area, practicing herbal medicine is a valid source of income.

Ngoyang area (2): Ngoyang is situated at around 15 km from Lolodorf on the Eséka-Lolodorf highway. Bakola pygmies who reputedly rely on herbal medications live in this area. The vegetation consists mainly of evergreen littoral humid rain forest with *Lophira alata*.

Kon-Yambetta area (3): This site is situated at around 150 km (northwest) from Yaoundé. This area has Guinea savannah-type flora and is inhabited by yambetta, and bamoun people to a lesser extent, who principally rely on herbs to treat their ailments.

Mbalmayo area (4): Mbalmayo is located at around 55 km (southern) from Yaoundé, and is the entering gate to the South region. It is a cosmopolitan area. In addition, the National School of Water and Forestry is in Mbalmayo, and has favoured the preservation of a forest where we conducted the survey.

The survey was preliminarily based on the selection of herbal practitioners for their ability to treat malaria and/or related symptoms using herbs. Subsequently, the identification of antimalarial plants with associated recipes was achieved through face-to-face interaction with practitioners.

2.2. Identification of herbal practitioners and selection to the survey

Patients identified through a brief information survey and villages' heads were interviewed to select herbal practitioners who treat malaria successfully within their communities and to include them in the study. In the four study sites, authorization to investigate within their community was sought from administrative authorities and/or village chiefs. They contributed in the identification of the most efficacious healers in treating malaria. To this end, small gatherings of people around the heads were organized during which the study purpose and potential benefits for the community were explained and herbal practitioners selected to the survey. All the participants to this survey have signed a memorandum of understanding to answer questions related to herbal practitioners and use of plants to treat malaria.

2.3. Identification of antimalarial Annonaceae plants

Subsequent interaction with the herbal practitioners included interviews and field collection of some samples. Healers were asked to provide information on herbs they use to treat malaria, emphasizing on plant parts used, modes of preparation and administration, and other plants or ingredients used in association. Specimens of plants identified through literature survey were also presented to herbal practitioners and their uses to treat malaria sought. When no specimen was available, vernacular and/or common names of plants to treat malaria-like symptoms. In the Water and Forestry School Mbalmayo, the survey was conducted with the head of the arboretum and forest schedule. Upon completion of the survey in each site, the ethnobotanist has signed the visitors' book in which he mentioned the subject of the visit.

This survey was conducted in strict respect of the Cameroonian current regulations of biodiversity protection and rural population's customary rights. During face-to-face interactions, the purpose and the procedure of the survey, as well as the expected benefits and rights were explained to villages' heads. The traditional herbal practitioners willing to collaborate on the study (or their interpreters) had signed the memorandum of understanding before further interactions. Interviews were conducted by Dr. Nole Tsabang, an ethnobotanist at the Institute for Medical Research and Medicinal Plants Study, Yaoundé, Cameroon, following a questionnaire purposely prepared. Interpreters were contracted when healers could not communicate in English or French. Voucher specimens of each plant were dried and deposited at the Cameroon National Herbarium and the Institute of Medical Research and Medicinal Plants Studies, Yaoundé.

Plants were collected with the aid of informants and identified first on the field of collection. This determination was later confirmed at the National Herbarium of Cameroon, Yaoundé. One ethnobotanical data sheet was completed for each recipe.

Plants that are used to treat malaria (diagnosed through simultaneous observation of fevers, headaches, chills, joint pains, and sweating) or at least two of these symptoms were searched. The approach adopted in this survey was previously used by Tsabang et al. in identifying Cameroonian plants with antidiabetic and antihypertensive properties (Tsabang et al., 2001, 2005; Tsabang, 2008).

3. Results

3.1. Repartition of informants

During the ethnopharmacological investigation we met 19/30 users of plants who agreed to work with us by providing information on plants that are used to treat malaria. Seven of them were traditional healers (Table 1). Inclusion of informants in this study depended on the signature of the MoU. Those who refused to sign the MoU were firstly, claiming that scientists were trying to steal their knowledge, and therefore could not cooperate; secondly, others rejected the offer claiming the allowance unsubstantial.

3.2. Knowledge about malaria

Malaria is known respectively in Ewondo and Bamileke tribes as "Titmeki" and "Nnect". Herbal practitioners who were interviewed identified malaria based on signs and symptoms that included fevers (100%), headaches (100%), chills (100%), joint pains (100%), sweating (90%), vomiting (55%), bitter taste in the mouth (50%), loss of appetite (45%) and spleen pains in children (40%) that are diagnosed by examinations of patients with upper left sided abdominal pain. The mode of transmission of the disease was well known and indicated as being via the bites of mosquitoes. Thus the people in the study areas had very good knowledge about how to identify malaria.

3.3. Botanical characteristics of Annonaceae plants

A total of 21 Annonaceae species belonging to 12 genera were reported being used in the preparation of herbal remedies against malaria and its symptoms in the four study sites. Of all the plants, 16/21 represented trees and 5/21 were shrubs; no herbaceous plants and no grasses were reported. Only 2 of the reported species were cultivated and 19 were wild. This indicates that the majority of plants being used in the study areas to treat malaria don't have some form of protection through cultivation (Table 2).

From Table 2, 11 species of Annonaceae plants were identified in Ngoyang (all wild), 10 in Mbalmayo (with 1 cultivated), 9 in Kon-Yambetta (with 2 cultivated), and 3 in Yaoundé and surroundings with 2 cultivated. *Annickia chlorantha* was identified in all the four sites. Ngoyang evergreen forest is undisturbed, and contains the majority of Annonaceae plants that have been identified. Indeed pygmies preserve more vegetation than the Bantu. The Arboretum of the Water and Forestry School of Mbalmayo is disturbed from high human pressure, though it still contains valuable plants species.

3.4. Place and frequency of collection of Annonaceae species

The majority (16/21) of the Annonaceae species used were collected in the forests (Atlantic evergreen rain forest and semideciduous rain forest), in home gardens (2/21), in cacao agroforests (1/21), and in savannah (1/21).

3.5. Ethnopharmacological recipes from plants studied

The ethnopharmacological characteristics of plants, parts used for the preparation of recipes, methods of preparation and administration are reported in Table 3.

From Table 3, 30 herbal recipes were identified to treat malaria and related symptoms in the four study sites. Two of these recipes were polyspecific.

The most used pharmaceutical form of recipe was decoction (74%). Only Pygmies were found to practice a form of sampling (scraping). The oral route of administration (97%) was found to be the most used. The stem bark was the most regularly used plant part (63%).

3.6. Routes of administration and dosage prescription of herbal remedies: description of recipes

94% of the herbal remedies used in the study areas were administered orally. Dosage prescriptions were adapted accordingly with age, where approximately a glassful (equivalent to 250 ml) or half a glassful (125 ml) were respectively prescribed three times daily

Table 1Distribution of informants.

Site	Traditional healers and other users	Sex (%)	Age range	
		Men	Women	
Ngoyang	6	6 (100%)	0 (0%)	30-60
Yaoundé and surroundings	4	1 (25%)	3 (75%)	40-65
Kon-Yambetta	4	3 (75%)	1 (25%)	40-65
Mbalmayo	5	2 (40%)	3 (60%)	40-54
Total	19	12 (63.16%)	7 (36.84%)	30-65

for adults and children. The treatments were supposed to be 3–10 days or continued until recovery.

The results obtained revealed two types of recipes: recipes reported to treat malaria and recipes treating one or two individual symptoms which can be caused by malaria. All these recipes are presented in Table 3.

3.6.1. Recipes reported to treat malaria

These recipes were clearly indicated by herbal practitioners as used to tackle malaria. Malaria was diagnosed via simultaneous observation of the more relevant clinical symptoms that are fevers, headaches, chills, joint pains, and sweating. Indeed, in much of malaria endemic Africa, these signs are closely related to malaria. The plants used for the preparation of recipes reported to treat malaria were Annickia chlorantha, Annona muricata, Annona senegalensis, Annona squamosa, Polyalthia suaveolens, Uvaria sp. and Polyceratocarpus sp. The description of these recipes is given in Table 3.

3.6.2. Recipes suspected to have antimalarial properties

These recipes were described as those used to treat individual symptoms that might be due to malaria. In this category, healers treat individual symptoms, which may or may not be caused by malaria. We therefore labeled them as recipes with possible antimalarial properties. These recipes are based on Anonidium mannii, Duguetia staudtii syn. Pachypodanthium mannii, Isolona hexaloba, Cleistopholis glauca, Cleistopholis patens, Cleistopholis staudtii, Hexalobus crispiflorus, Monodora brevipes, Monodora myristica, Monodora tenuifolia, Xylopia aethiopica, Xylopia hypolampra, Xylopia parviflora and Xylopia staudtii.

Table 2

Distribution of identified Annonaceae plants.

Annonaceae species	Locality				Nature of plant	Place of collection
	Ydé & surr. Ngo. Kon-Y.		Mbal.			
1. Annickia chlorantha (Oliv.) Setten & Maas	1	1	1	1	Tree	Forest
2. Annona muricata Linn.	1	0	1	1	Shrub	Cultivated
3. Annona senegalensis Pers.	0	0	1	0	Shrub	Savannah
4. Annona squamosa Linn.	1	0	1	0	Shrub	Cultivated
5. Anonidium mannii (Oliv.) Engl. & Diels	0	1	0	1	Tree	Forest
6. Cleistopholis glauca Pierre ex Engl. & Diels	0	1	1	0	Tree	Forest
7. Cleistopholis patens (Benth.) Engler & Diels	0	1	0	0	Tree	Forest
8. Cleistopholis staudtii Engler & Diels	0	1	1	0	Tree	Forest
9. Duguetia staudtii (Engl. & Diels) Chatrou	0	1	0	1	Tree	Forest
10. Hexalobus crispiflorus A. Rich.	0	1	0	0	Tree	Forest
11. Isolona hexaloba (Pierre) Engler & Diels	0	1	0	0	Tree	Forest
12. Monodora brevipes Benth.	0	1	0	0	Tree	Forest
13. Monodora myristica (Gaertn.) Dunal.)	0	0	1	0	Tree	Agroforest
14. Monodora tenuifolia Benth.	0	0	0	1	Tree	Forest
15. Polyalthia suaveolens Engl. & Diels.	0	1	0	1	Tree	Forest
16. Uvaria sp.	0	0	1	0	Shrub	Forest
17. Polyceratocarpus sp.	0	0	1	0	Shrub	Forest
18. Xylopia aethiopica (Dunal) A. Rich	0	0	0	1	Tree	Forest
19. Xylopia hypolampra Mildbr. & Diels	0	1	0	1	Tree	Forest
20. Xylopia parviflora (A. Rich.) Benth.	0	0	0	1	Tree	Forest
21. Xylopia staudtii Engl. & Diels	0	0	0	1	Tree	Arboretum

Ydé & surr. = Yaoundé and its surroundings; Ngo. = Ngoyang; Kon-Y. = Kon-Yambetta; Mbal. = Mbalmayo; 1 = species recorded in the site; 0 = unrecorded species in the site.

4. Discussion

The present survey has provided information about 21 species of Annonaceae plants used in the treatment of malaria in four areas of Cameroon. Malaria is a serious health issue in these four areas and *Annickia chlorantha* was found to be the main plant used all across the four sites. The two polyspecific recipes based on this species were said to show more potency. In fact they consisted of several plants, indicating that at least one of these plants contains compounds with potent antimalarial activity. The efficiency of this species underlies its potential as source of antimalarial agents, and should be subjected to further scientific investigations (Heinrich et al., 2009).

The Annonaceae plants described in this study were mostly collected in forests whereas in Ghana, the majority of the plants used against malaria are collected from neighborhood of habitations and in forest reserve areas (Asase et al., 2005). In Msambweni and Kwale Districts in Kenya, plants were collected from community lands (Muthaura et al., 2007; Nguta et al., 2010). Whether plants are collected near homesteads, in forest reserves or elsewhere in the bush, it is important to develop strategies to ensure their sustainable use and conservation. Of note, the bark of Annickia chlorantha is sold in local, regional and national markets. Therefore, accurate information is hardly available on the time of the year and/or day as well as place of plant collection. Plant parts should therefore be harvested when needed using simple tools such as knives or machetes without rending the bark. Axes and saws are not recommended for this practice. In the Bakola pygmies area, only the required amount of plant materials was usually collected for treatment and therefore the probability of overharvesting plant materials was insignificant in that group. From studies in Ethiopia, it is shown that harvesting

Table 3

Methods of preparation and administration of herbal recipes.

Species, common name, vernacular name and (dialect)	Parts used and recipes	Methods of preparation	Route of administration	Posology and duration of treatment	Frequency	Major symptoms and other diseases treated
Recipes reported to treat malaria 1. <i>Annickia chlorantha</i> (Oliv.); yellow Moambe; MPOL (Pygmies Bakola)	1. Stem bark	Decoction of 500 g of stem bark removed by scraping with a machete in 3 l of water for 20 min	Orally	Take 250 ml decoction 3 times daily for 15 days	3	Aches, wounds, boils, vomitin yellow bitter, fever, chills, sor spleen in children, and hepatitis
	^a 2. Stem bark + other plants	Decoction of a mixture of 300 g of stem bark of each of these plants: Annickia chlorantha, Rauvolfia vomitoria, Fagara macrophylla in 41 of water for 20 min	Orally	Take 250 ml of decoction 3 times a day for 10 days	1	
		Decoction of a mixture of 300 g of stem bark of each of these plants: Annickia chlorantha, Rauvolfia vomitoria, Fagara macrophylla and Nauclea latifolia in 41 of water for 20 min	Orally	Take 250 ml of decoction 3 times a day for 10 days	1	
2. Annona muricata Linn.; Soursop (English); Sabasaba 'Yambetta)	3. Leaves	Decoction of a handful of leaves in 31 of water for 20 min	Orally	Take 250 ml of decoction once a day for 7 days	1	Aches, backpains
B. Annona senegalensis Pers.; Wild soursop (English); Kedahan (Yambetta)	4. Stem bark	Decoction of 500 g of roots stem bark in 3 l of water for 20 min	Orally	Drink 250 ml of a decoction once a day for 15 days	1	Vomiting, muscle aches, tiredness, jaundice, fever, convulsions, respiratory
	^a 5. Young leafy stems	Decoction of 100 g of young leaves of Annona senegalensis, with 100 g young leaves of Piptostigma thonningii, 100 g of leaves of Senna alata, 100 g of Chrysanthellum americana, 100 g of Lippia multiflora, 300 g of Terminalia glaucescens, 300 g root stem of Nauclea latifolia, 100 g of Ocimum gratissimum in 51 of water. Reduce by boiling the decoction to 31. The roots of Nauclea latifolia should be harvested at sunrise or sunset	Orally	Drink one glass (250 ml) morning and evening	1	infections, snake bites
I. Annona squamosa Linn.; Sugar apple (English); Kedahan Yambetta)	6. Leaves	Decoction of 150 g of leaves in 3 l of water for 20 min	Orally	Drink 250 ml of decoction 2 times daily for 10 days	1	Vomiting, muscle aches, tiredness, abscesses, fever, convulsions, digestive disorders and skin diseases
5. Polyalthia suaveolens Engl. & Diels; Otungui; Ntounga Pygmies Bakola)	7. Stem bark	Decoction of 500 g of stem bark removed by scraping with a machete in 3 l of water for 20 min	Orally	Drink 250 ml of decoction 3 times daily for 15 days	1	Fever, jaundice, epilepsy, join pains, headache
	8. Fruits	Decoction of one serrated fruit in 21 of water for 15 min	Orally	Drink 250 ml of decoction 3 times daily for 7 days	1	
6. Uvaria sp.; Nosonaback Yambetta)	9. Stem bark	Decoction of 500 g of stem bark collected from scraping with machete in 3 l of water, evaporating the water to 1/2	Orally	Drink 250 ml of decoction 3 times a day	1	Typhoid and yellow fever, headache, epilepsy

Table 3 (Continued)

Species, common name, vernacular name and (dialect)	Parts used and recipes	Methods of preparation	Route of administration	Posology and duration of treatment	Frequency	Major symptoms and other diseases treated
7. <i>Polyceratocarpus</i> sp.; Nosonaback (Yambetta)	10. Stem bark	Decoction of 500 g of stem bark collected from scraping with machete in 3 l of water, evaporating the water to 1/2	Orally	Drink 250 ml of decoction 3 times a day	1	Typhoid and yellow fever, headache, epilepsy
Recipes used to treat at least two s	ymptoms which may be caused b	by malaria				
8. Anonidium mannii Gaertm (D. Oliver) Engl.& Diels; Ebome; Npole Wapo'o (Pygmies Bakola), Ebome Afan (Ewondo and Bulu)	11. Stem bark	Decoction of 500 g of stem bark from scraping with machete in 31 of water, evaporating the decoction to 2/3	Orally	Drink 250 ml of decoction 3 times a day for 10 days	3	Childhood illness (diaper rash, measles, diarrhea, enlarged spleen), fever, amoeba (STD, candidiasis, gonorrhea, etc.), promotion of breast-milk
			Vaginally	Use the decoction to the personal hygiene	1	production
9. <i>Cleistopholis glauca</i> Pierre ex Engl.& Diels; Sobu; Wombo (Pygmies Bakola), Avom (Ewondo and Bulu)	12. Stem bark	Decoction of 500 g of stem bark obtained from scraping with machete in 21 of water for 24 h, or 100 g of leaves in 31 of water for 20 min	Orally	Drink 250 ml of decoction once a day for 7 days	3	Fever, muscle ache, arthritis, wounds, boils
10. Cleistopholis patens (Benth.) Engl.& Diels; Salt-and-oil tree (English); Wombo (Pygmies Bakola), Avom (Ewondo and Bulu)	13. Stem bark	Decoction of 500 g of stem bark obtained from scraping with machete in 2 l of water for 24 h, or 100 g of leaves in 3 l of water for 20 min	Orally	Drink 250 ml of decoction once a day for 7 days	3	Fever, muscle ache, arthritis, wounds, boils
11. <i>Cleistopholis staudtii</i> (Engl.& Diels) Engl.& Diels; Sobu; Wombo (Pygmies Bakola), Avom (Ewondo and Bulu)	14. Stem bark	Decoction of 500 g of stem bark obtained from scraping with machete in 2 l of water for 24 h, or 100 g of leaves in 3 l of water for 20 min	Orally	Drink 250 ml of decoction once a day for 7 days	3	Fever, muscle ache, arthritis, wounds, boils
12. Duguetia staudtii (Engl.& Diels) Chatrou syn. Pachypodanthium mannii; Ntom; Ntoma (Pygmies Bakola), Ntom (Ewondo and Bulu)	15. Stem bark	Maceration of 500 g of stem bark in 31 of water under sunlight for 6 h	Orally	Drink 250 ml every 12 h for 3 days	1	Vomiting, asthenia, arthritis, headache, cough, stiffness, skinache
		Decoction of 500 g of stem bark obtained from scraping with machete, or 100 g of leaves in 31 of water for 20 min	Head bath	Drink 250 ml of decoction twice a day for 15 days	3	
13. Hexalobus crispiflorus A. Rich; Owe; Lefondja (Pygmies Bakola), Avom (Ewondo and Bulu)	16. Stem bark	Decoction of 500 g of stem bark obtained from scraping with machete, or 100 g of leaves in 31 of water for 20 min	Orally	Drink 250 ml of decoction 3 times daily for 7 days	3	Convulsion, fever, muscle ache and arthritis, wounds, boils
14. Isolona hexaloba Pierre (Engl. & Diels); Nding; Lesondje (Pygmies Bakola), Nding or Nom Ntom (Ewondo)	17. Stem bark	Infusion of 500 g of stem bark obtained from scraping with machete in 2 l of water for 24 h	Orally	Drink 250 ml of infusion once every 2 days during 2 consecutive weeks	1	Wounds, boils, fever

Table 3 (Continued)

Species, common name, vernacular name and (dialect)	Parts used and recipes	Methods of preparation	Route of administration	Posology and duration of treatment	Frequency	Major symptoms and other diseases treated
15. <i>Monodora brevipes</i> Benth.; yellow-flowered; Pio (Pygmies Bakola), Nom Akwi grandes feuilles (Ewondo)	18. Stem bark	Decoction of one tea spoonful of stem bark and or the same amount of stem bark powder in 250 ml of water	Orally	Drink 250 ml of decoction twice a day for 10 days	2	Fever, cough
	19. Fruits (powder)	Infusion of one tea spoonful of ground dry seeds powder in 250 ml of water	Orally	Drink 250 ml of infusion twice a day for 10 days	2	
16. <i>Monodora myristica</i> (Gaertn.) Dunal; Calabash nufmeg; Bongo (Yambetta), Akwi (Ewondo)	20. Stem bark	Decoction of 1000 g of stem bark in 41 of water for 20 min	Orally	Take a purge every 2 days	1	Sore groin in women, joint pains, headache
	21. Fruits (powder)	Infusion of 1000 g of seed powder in 41 of water	Orally	Take a purge every two days	1	
17. <i>Monodora tenuifolia</i> Benth.; <i>Monodora</i> with thin leaves; Ebome osso (Ewondo)	22. Stem bark	Decoction of 500 g of stem bark powder in 3 l of water for 20 min	Orally	Drink 250 ml of decoction 3 times a day for 7 days	1	Joint and muscle pain, headache and promotion of breast milk production
	23. Fruits (powder)	Infusion of 500 g of dry seed powder in 31 of water	Orally	Drink 250 ml of infusion 3 times a day for 7 days	1	I CONTRACTOR
18. <i>Xylopia aethiopica</i> (Dunal) A. Rich; Ethiopian pepper; Akwi (Ewondo)	24.Stem bark	Decoction of one teaspoon of crushed dried stem bark in 1 l of water	Orally	Drink 250 ml 2 times daily for 7 days	1	Fruits are used against ache fever and as spice
	25. Fruits	Maceration of one teaspoon of crushed dried fruits in 1 l of water	Orally	Drink 250 ml 2 times daily for 7 days	1	
9. Xylopia hypolampra Mildbr. 2 Diels; Sedhiou pepper; Ikanla (Pygmies Bakola), Nom .kwi (Ewondo)	26. Stem bark	Decoction of one teaspoon of crushed dried stem bark in 500 ml of water	Orally	Drink 250 ml of the resulting sauce 2 times daily for 15 days	1	Fever, chills
	27. Fruits	Maceration of one teaspoon of crushed dried fruits in 500 ml of water	Orally	Drink 250 ml of the resulting sauce 2 times daily for 15 days	1	
20. Xylopia parviflora (A. Rich) Benth.; Ashako; Odjobi (Ewondo)	28. Stem bark	Decoction of 1000 g of stem bark in 31 of water for 20 min	Orally	Drink 250 ml of decoction 3 times daily for 7 days	1	Sore groin in women, abscesses, lung infections, body aches, headache, fever
	29. Fruits (powder)	Infusion of one teaspoon of crushed dried fruits in 250 ml of water	Orally	Take 2 times daily for 10 days	1	
21. <i>Xylopia staudtii</i> Engl. & Diels; Odjwe; Nkanla (Pygmies Bakola), Nom Akwi (Ewondo)	30. Fruits	Maceration of one teaspoon of crushed dried fruits in 500 ml water	Orally	Drink 250 ml of the mixture 2 times daily for 15 days	2	Fever, chills
Fotal: 21 plants	30 Recipes	31 modes of preparation	32 routes of administration			

^a Polyspecific recipes.

time can be an important issue and some plants only exhibit their full therapeutic effect if they are collected early in the morning or during a certain season (Abebe, 1984). Also, according to Prance (1994), the potency of vegetal remedies could differ according to the method of extraction, place and time of harvesting plant materials as well as other environmental factors like soil, climate, use of chemical fertilizers, altitude, etc.

This indicates that the potency of antimalarial Annonaceae remedies used in the study areas could greatly vary depending on the time and place where the plant materials were harvested for use. This was confirmed by a study of time factor by Tsabang in the Phytotherapy of diabetes and/or hypertension (unpublished results).

The majority of the herbal preparations identified in this study involved decoction and oral administration. Accordingly, in a similar study in the Far-North region of Cameroon, the pharmaceutical form and route of administration were found to be the same (Soatoing et al., 2011).

The fact that stem bark represented 63% of the parts used may be related to its availability and accessibility to people, and to its high content of antimalarial agents. In the contrary, fresh leaves were found to be the most commonly plant part used in herbal remedies for the treatment of malaria in the Wechiau Community Hippopotamus Sanctuary in Ghana (Asase et al., 2005) and the Msambweni District in Kenya (Nguta et al., 2010). Overall, plant parts were similarly used in fresh state in all these studies.

The problems associated with dosage prescription in the use of herbal remedies for the treatment of malaria have been highlighted by a number of authors (Asase et al., 2005; Muthaura et al., 2007; Tabuti, 2008; Nguta et al., 2010). The interviewees reported that their herbal remedies had no side effects but this was anticipated as most traditional healers do not, broadly speaking, know of the specific side effects of their herbal remedies. Similar observations have been made in the Budiope County in Uganda (Tabuti, 2008), some districts in Tanzania (Gessler et al., 1995) and the Dangme West District of Ghana (Asase et al., 2010).

To elaborate some medicinal preparations, a mixture of different plants was used in some few cases. A similar approach was described for antimalarial preparations in Cuba (Hernandez and Volpato, 2004), Trinidad (Clement et al., 2005) and Dominican Republic (Vandebroek et al., 2010) where the inhabitants also use mixture of plants, purposely to increase the bioactivity.

Literature sources in Cameroon and Africa show that Anonidium mannii, Annickia chlorantha and Duguetia staudtii are used for the treatment of malaria by Bulu of Western Dja Reserve (Betti, 2006), pygmies Baka and Badjoue of East region (Tsabang Nole, personal communication) and the ethnic groups (Ejegham, Bulu, Oroko) in Korup (Duncan et al., 1989). Another recent study has revealed that many of the species of the Annonaceae growing in the Mount Cameroon region are of significant ethnobotanical importance (Focho et al., 2010). According to these authors, many species described in this work (Annickia chlorantha, Annona senegalensis, Monodora myristica, Monodora tenuifolia, Uvaria sp., Xylopia staudtii, Polyceratocarpus sp.) are used to treat many symptoms that might be related to malaria, including fevers, vomiting, fatigue, joint pains, headache, and stomach ache. But no direct indication was made concerning their use against malaria.

In the Democratic Republic of Congo, some Annonaceae plants described in this study are used to treat malaria or associated symptoms. These plants are: *Annickia chlorantha, Polyalthia suaveolens, Monodora myristica* and *Duguetia staudtii* (Bouquet, 1969; Adjanohoun et al., 1996). Overall, *Anonidium mannii, Annickia chlorantha, Duguetia staudtii, Polyalthia suaveolens*, and *Monodora myristica* investigated in this work were found to be used to treat malaria in other parts of Africa.

Furthermore, complementary information was documented through this investigation on the ethnopharmacological use of Annonaceae plants to treat malaria and/or symptoms in Cameroon, including but not limited to Annona muricata, Annona senegalensis, Annona squamosa, Annonidium mannii, Hexalobus crispiflorus, Isolona hexaloba, Monodora myristica, Monodora brevipes, Monodora tenuifolia, Polyceratocarpus sp., Xylopia aethiopica, Xylopia hypolampra, Xylopia parviflora, Xylopia staudtii, Uvaria sp. Meanwhile, some of these species are reported to be traditionally used elsewhere to treat malaria, including Annona muricata (Di Stasi et al., 1994; Leaman et al., 1995; Coe and Anderson, 1996; Pinto et al., 2005), Annona senegalensis (Chhabra et al., 1987; Bugmann, 2000), Annona squamosa (Comerford, 1996), Xylopia aethiopica (Mulangu Binzambal, 1999).

Laboratory studies have previously reported the antiplasmodial activity of extracts from many of these plants growing in Cameroon. Amongst the extracts with activity, many were volatile extracts from Cleistopholis patens (Boyom et al., 2011b), Xylopia aethiopica, Hexalobus crispiflorus, (Boyom et al., 2003), Xylopia staudtii, Xylopia aethiopica, Xylopia parviflora, Monodora brevipes, Monodora myristica, Polyalthia suaveolens, Annickia chlorantha, Annona senegalensis (Boyom, 2004). Non-volatile extracts from Annickia chlorantha, Annona muricata, Anonidium mannii, Monodora myristica, Polyalthia suaveolens, Uvaria banmanni, Xylopia aethiopica, and Xylopia parviflora also showed potency against Plasmodium falciparum in vitro (Boyom et al., 2009, 2011a). Some of these Annonaceae species from different origins were also investigated for antimalarial activity, including Annona muricata (Leaman et al., 1995; Bidla et al., 2004), Annona squamosa (Bagavan et al., 2011; Johns et al., 2011), Xylopia sp. (Jenett-Siems et al., 1999; Akam et al., 2005). Thus, ethnobotanical and pharmacological data support the relevance of Annonaceae plants as a promising source of potent antimalarial agents. Further detailed studies are therefore required to determine the phytochemical and pharmacological properties of these plants. These studies if promising will be the starting point of further development of alternative drugs that will help tackle the developing resistance of Plasmodium falciparum to currently used Artemisininbased Combination Therapies (ACTs) (Dondorp et al., 2009).

Moreover, in the framework of the improvement and promotion of the local traditional knowledge in using herbs against malaria, the pharmacological, phytochemical and toxicological profile of the herbal remedies used need to be investigated in order to ensure the effective treatment for malaria as well as the safety of people.

Overall, this survey underlines that seven of the identified species of Annonaceae were effectively used to treat malaria, while 14 were used to treat symptoms that might be related to malaria. In most of the tropical malaria-endemic countries, the symptoms described are mostly associated with malaria. Therefore, there is a realistic potential that these effective plants contain compounds with antimalarial potency.

5. Concluding remarks

The present study has revealed that people in the four study sites have a very good knowledge of using Annonaceae species to treat malaria. Herbal remedies commonly used for the treatment of malaria were affordable and easily accessible in their settings.

The plant harvesting technique used by Bakola pygmies that consisted to scrape superficially the stem bark of the tree downwards with a knife or machete was found to be apparently the best practice. Indeed, scraping gives more ability to the plant to quickly regenerate the bark that is one of the critical organs for plant survival.

Previous studies have allowed us to highlight some similarities in the use of certain anti-malaria plants species. Thus *Annickia* *chlorantha* is the most sought Annonaceae plant identified in this study.

On another hand, a study on the characterization of Annonaceae using phylogeny techniques have shown that the species that has been called *Annickia chlorantha* for a long time, in fact represents two species. In this study *Annickia chlorantha* and *Annickia affinis* are distinguished as two different species, *Annickia chlorantha* being the rarer species (Couvreur et al., 2009). Thus, plants described in the four study sites as *Annickia chlorantha* most likely represent the two species.

This study has documented significant information on Annonaceae species that are used in the preparation of herbal remedies for the treatment of malaria and/or symptoms that might be related to malaria in the study sites. Over 5 of the species encountered in the study areas have been previously documented elsewhere in Africa for their use in the treatment of malaria. This highlights their wide range recognition in African traditional knowledge as credible sources of therapy against malaria. The pharmacological, phytochemical and toxicological properties of the herbal remedies used should be investigated in detail with respect to their origins and modes of preparation. Furthermore, information gathered from traditional knowledge on plant collection and extraction should subsequently guide the designing of laboratory procedures. This information will be also useful in the proper selection of active plants for cultivation and subsequent conservation in the study area.

The treatment of malaria symptoms was also highlighted in this study as an important approach for the identification of antimalarial plants. This documentation will contribute to the development of effective management strategies for malaria control, especially in Sub-Saharan Africa where the burden of the disease is very high.

Acknowledgements

The successful completion of this study was a summary of contributions from the Bakola Pygmies, Yambetta, and Yaoundé people and healers, and the staff of the School for Water and Forests, Mbalmayo, Cameroon. The authors are very grateful for their hospitality during fieldwork and permission to publish the data. We are mostly grateful to Chief Nkoro of Pygmies Bakola community, Chief Youkoulouk of Bamoko community, Deputy sub-prefect of Kon-Yambetta, Badje Badje of Water and Forests School of Mbalmayo, and Jean Paul Ghogue of the National Herbarium of Cameroon.

This work was made possible by funding from the World Health Organization, through the Initiative for Research on Tropical Diseases and the Multilateral Initiative against Malaria (MIM/TDR) (A80689). Thanks to the anonymous reviewers for a critical review of this paper.

References

- Abebe, W., 1984. Traditional pharmaceutical practice in Gondar region, Northwestern Ethiopia. Journal of Ethnopharmacology 11, 33–47.
- Adjanohoun, J.E., Aboubakar, N., Dramane, K., Ebot, M.E., Ekpere, J.A., Enoworock, E.G., Focho, D., Gbile, Z.O., Kamanyi, A., Kamsu, K.J., Keita, A., Mbenkum, T., Mbi, C.N., Mbiele, A.C., Mbome, J.C., Muberu, N.K., Nancy, W.L., Kongmeneck, B., Satabie, B., Sofowor, A., Tamze, V., Wirmum, C.K., 1996. Traditional medicine and pharmamacopoeia: contribution to ethnobotanical and floristic studies in Cameroon. In: Organisation of African Unity (Ed.), Scientific Technical and Research Comity, Addis Ababa.
- Akam, T., Yong, J., Fanso-Free, S., 2005. Antiplasmodial agents from Xylopia parviflora seeds. MIM Proceedings, Acta Tropica 95 (Suppl. 1), 197–198.
- Asase, A., Akwetey, G.A., Achel, D.G., 2010. Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West District of Ghana. Journal of Ethnopharmacology 129, 367–376.
- Asase, A., Oteng, Y.A.A., Odamtten, G.T., Simmonds, M.S.J., 2005. Ethnobotanical study of some Ghanaian antimalarial plants. Journal of Ethnopharmacology 99, 273–279.

- Bagavan, A., Rahuman, A.A., Kaushik, N.K., Sahal, D., 2011. In vitro antimalarial activity of medicinal plant extracts against *Plasmodium falciparum*. Parasitology Research 108, 15–22.
- Betti, J.L., 2006. Usages populaires des plantes médicinales chez les Bulu à l'Ouest de la réserve du Djà Cameroun. Revue Médicale de la Pharmacopée Africaine 19, 93–106.
- Bidla, G., Titanji, V.P.K., Joko, B., Ghazali, G.E., Bolad, A., Berzins, K., 2004. Antiplasmodial activity of seven plants used in African folk medicine. Indian Journal of Pharmacology 36, 245–246.
- Bouquet, A., 1969. Féticheurs et Médecines Traditionnelles du Congo (Brazzaville). O.R.S.T.O.M., 250 pp.
- Boyom, F.F., Tsouh, F.P.V., Tchokouaha, Y.L.R., Ngoutane, M.A., Madiesse, K.E., Mbacham, F.W., Tsamo, E., Amvam, Z.P.H., Gut, J., Rosenthal, P.J., 2011a. Potent antiplasmodial extracts from Cameroonian Annonaceae. Journal of Ethnopharmacology 134, 717–724.
- Boyom, F.F., Ngouana, V., Madiesse, K.E.A., Amvam, Z.P.H., Menut, C., Bessiere, J.M., Gut, J., Rosenthal, P.J., 2011b. Antiplasmodial volatile extracts from *Cleistopholis patens* Engl and Diels and *Uvariastrum pierreanum* Engl. (Engl. and Diels). Parasitology Research 108, 1211–1217.
- Boyom, F.F., Madiesse, K.E., Tepongning, R., Ngouana, V., Mbacham, W.F., Tsamo, E., Amvam Zollo, P.H., Gut, J., Rosenthal, P.J., 2009. Antiplasmodial activity of extracts from seven medicinal plants used in malaria treatment in Cameroon. Journal of Ethnopharmacology 123, 483–488.
- Boyom, F.F., 2004. Huiles essentielles d'Annonacées Camerounaises: Analyses chimiques, Activité antioxydantes et Antiplasmodiales. Doctorat d'Etat Thesis in Biochemistry, University of Yaoundé I, p. 164.
- Boyom, F.F., Ngouana, V., Amvam Zollo, P.H., Menut, C., Bessiere, J.M., Gut, J., Rosenthal, P.J., 2003. Composition and antiplasmodial activities of essential oils from some Cameroonian medicinal plants. Phytochemistry 64, 1269–1275.
- Bugmann, N., 2000. Le concept du paludisme, l'usage et l'efficacité in vivo de trois traitements traditionnels antipalustres dans la région de Dori, Burkina Faso. Doctoral Thesis, Faculty of Medicine, University of Basel.
- Chhabra, S.C., Mahunnah, R.L.A., Mshiu, E.N., 1987. Plants used in traditional medicine in eastern Tanzania. I. Pteridophytes and Angiosperms (Acanthaceae to Canellaceae). Journal of Ethnopharmacology 21, 253–277.
- Clement, N., Williams, A., Aranda, D., Chase, R., Watson, N., Mohamened, R., Stubbs, O., Williamson, D., 2005. Medicinal herbs used among asthmatic patients attending a specialty care facility in Trinidad, BMC. Complementary and Alternative Medicine 5, 3.
- Coe, F.G., Anderson, G.J., 1996. Ethnobotany of the Garífuna of Eastern Nicaragua. Economic Botany 50, 71–107.
- Comerford, S.C., 1996. Medicinal plants of two Mayan healers from San Andrés, Petén, Guatemala. Economic Botany 50, 327–336.
- Couvreur, T.L.P., Van der Ham, R.W.J.M., Mbele, Y.M., Mbago, F.M., Johnson, D.M., 2009. Molecular and morphological characterization of a new monotypic genus of Annonaceae, *Mwasumbia*, from Tanzania. Systematic Botany 34, 266–276.
- Di Stasi, L.C., Hiruma, C.A., Guimaraes, E.M., Santos, C.M., 1994. Medicinal plants popularly used in Brazilian Amazon. Fitoterapia 65, 529–540.
- Dondorp, A.M., Nosten, F., Yi, P., Das, D., Phyo, A.P., Tarning, J., Lwin, K.M., et al., 2009. Artemisinin resistance in *Plasmodium falciparum* Malaria. New England Journal of Medicine 361, 455–467.
- Duncan, W.T., Jane, M.T., Wendy, A.B., Funki, T.M., 1989. Korup Ethnobotany Survey. The World Wide Fund for Nature. Panda House Weyside Park Godalming, Survey, UK, 154 pp.
- Focho, D.A., Egbe, E.A., Chuyong, G.B., Fongod, A.G.N., Fonge, B.A., Ndam, W.T., Youssoufa, B.M., 2010. An ethnobotanical investigation of the Annonaceae on Mount Cameroon. Journal of Medicinal Plants Research 4, 2148–2158.
- Gessler, M.C., Msuya, D.E., Nkunya, M.H.H., Mwasumbi, L.B., Schär, A., Heinrich, M., Tanner, M., 1995. Traditional healers in Tanzania: the treatment of malaria with plant remedies. Journal of Ethnopharmacology 48, 131–144.
- Hay, S.I., Okiro, E.A., Gething, P.W., Patil, A.P., Tatem, A.J., Guerra, C.A., Snow, R.W., 2010. Estimating the global clinical burden of Plasmodium falciparum malaria in 2007. PLoS Medicine 7, e1000290, doi:10.1371/journal.pmed.1000290.
- Heinrich, M., Edwards, S., Moerman, D.E., Leonti, M., 2009. Ethnopharmacological field studies; a critical assessment of their conceptual basis and methods. Journal of Ethnopharmacology 124, 1–17.
- Hernandez, C., Volpato, G., 2004. Herbal mixture in the traditional medicine of Eastern Cuba. Journal of Ethnopharmacology 90, 293–316.
- Jenett-Siems, K., Mockenhaupt, F.P., Bienzle, U., Gupta, M.P., Eich, E., 1999. In vitro antiplasmodial activity of Central American medicinal plants. Tropical Medicine and International Health 4, 611–615.
- Johns, T., Windust, A., Jurgens, T., Mansor, S.M., 2011. Antimalarial alkaloids isolated from Annona squamosa. Phytopharmacology 1, 49–53.
- Leaman, D.J., Arnason, J.T., Yusul, R., Sangat-Roemantyo, H., Soedjito, H., Angerhofer, C.K., Pezzuto, J.M., 1995. Malaria remedies of the Kenyah of the Apo Kayan, East Kalimantan, Indonesian Borneo: a quantitative assessment of local consensus as an indicator of biological efficacy. Journal of Ethnopharmacology 49, 1–16.
- Mulangu Binzambal, O., 1999. Antimalarial plants used in the Democratic Republic of Congo. Poster: First International Meeting of the Research Initiative on Traditional Antimalarials. http://mim.nih.gov/english/ partnerships/ritam_program.pdf.
- Muthaura, C.N., Rukunga, G.M., Chhabra, S.C., Mungai, G.M., Njagi, E.N.M., 2007. Traditional antimalarial phytotherapy remedies used by the Kwale community of the Keyan Coast. Journal of Ethnopharmacology 144, 377–386.

- Nguta, J.M., Mbaria, J.M., Gakuya, D.W., Gathumbi, P.K., Kiama, S.G., 2010. Antimalarial herbal remedies of Msambweni, Kenya. Journal of Ethnopharmacology 128, 424–432.
- Pinto, A.C., Andrade, S.R., Ferreira, F.R., Kinpara, D.I., 2005. Annona Species. International Center for Underutilised Crops, University of Southampton, Southampton S0171BJ, UK, pp. 1–281.
- Prance, G.T., 1994. Introduction. In: Prance, G.T., Chadwick, D.J., Marsh, J. (Eds.), Ethnobotany and the Search for New Drugs. Ciba Foundation Symposium 185. John, Wiley and Sons, Chichester, England, pp. 57–90.
- Soatoing, P., Vroumsia, T., Tchobsala, T.F., Njan, N.A., Messi, J., 2011. Medicinal plants used in traditional treatment of malaria in Cameroon. Journal of Ecology and Natural Environment 3, 104–117.
- Tabuti, J.R.S., 2008. Herbal medicines used in the treatment of malaria in Budiope County, Uganda. Journal of Ethnopharmacology 116, 33–42.
- Tsabang N., 2008. Etude ethnobotanique des plantes à vertus antidiabétiques et/ou antihypertensives au Cameroun. PhD Thesis in Ethnobotany, University of Yaoundé 1, Cameroon, 318 pp.
- Tsabang, N., Nkongmeneck, B.A., Lejoly, J., Nguenang, G.M., Fongnzossie, F.E., Koueke, P., Biyiti, T.V., Foko, J., Mbitta, M.J.C.H., Atangana, J., 2005. Médécine traditionnelle: une méthode d'indentification des diabétiques et/ou des hypertendus et des plantes pouvant améliorer leur état de santé. Cameroon Journal of Ethnobotany 1, 53–59.
- Tsabang, N., Nkongmeneck, B.A., Zapfack, L., Nzooh, D., Nguenang, G.M., Lando, G., Carlson, T.J., Porter, P.L., 2001. Inventaire des plantes à vertus antidiabétiques dans la région de Yaoundé au Cameroun. Revue Médicale de la Pharmacopée Africaine 15, 87–94.
- Vandebroek, I., Balick, M.J., Ososki, A., Kronenberg, F., Yukes, J., Wade, C., Jimenez, F., Peguero, B., Castillo, D., 2010. The importance of *Botellas* and other plant mixture in Dominican traditional medicine. Journal of Ethnopharmacology 128, 20–41.
- World Malaria Report, 2010. WHO Press Geneva, Switzerland 3, pp. 1084–1092.