

# **Ethnobotanical Study Of Medicinal Plants In The Fight Against Buruli Ulcer In The Maritime Region Of Togo**

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## **Abstract**

**Background:** In Togo, few data are available on the traditional treatment of Buruli ulcer, so this study aim to inventory and identify the plants

and recipes included in the herbal medicines in the maritime region where this disease is more common. **Method:** The methodology was based on ethnobotanical semi-structural individual interviews of 16 traditional healers in the maritime region. The Data were collected and analyzed in Microsoft excel 2013. **Results:** 10 of the traditional healers were male and the average age of all the respondents was  $42.31 \pm 4.24$  years. A total of 27 plant species belonging to 20 families were identified. The most represented families were: Caesalpiniaceae with 3 species, Euphorbiaceae, Apocynaceae, Asteraceae, Sapindaceae and Annonaceae with 2 species each. In the preparation methods, the decoction is the most used (37%), followed by the black powdered incorporated in an ointment (24%), the maceration (15%), the infusion (12%), the spraying (6%) and poultices (6%). These modes of preparation, were administered mainly externally by massage, disinfection of the lesion and orally. The most used parts of the plants were leaves (48%), roots (21%), and bark (10%). The species with the highest frequency of use were *Blighia sapida* KD Koenig, *Amaranthus spinosus* L, *Ocimum canum* Sims, *Piliostigma thonningii* (Schum.) Milne-Redh, *Aloe vera* L, *Anacardium occidentale* L, *Paullinia pinnata* L and *Xylopiya aethiopica* (Dunal) A.Rich. **Conclusion:** By highlighting these plant species of interest, this study has an applicability in therapeutic innovation. It can contribute to the chemical, pharmacological and clinical evaluations of their molecules leading to development of phytomedicines to produce new drugs to strengthen the already existing therapeutic arsenal.

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**Keywords:** Buruli ulcer, Plants, Traditional treatment, Togo Maritime Region

## Introduction

Buruli Ulcer (BU) is a skin disease caused by a mycobacterium called *Mycobacterium ulcerans*. The outbreaks are geographically almost always circumscribed around an aquatic ecosystem (river, artificial or natural lake, marsh area, irrigation system) (Maman *et al*, 2018; Portaels *et al*, 2008). In 2019, Buruli ulcer has been identified by the World Health Organization (WHO) in more than 30 countries on several continents (Africa, America, Asia, and Oceania) (OMS, 2019). In Africa 10 countries have reported cases of BU to WHO, including 4 in west Africa, notably Ghana, Côte d'Ivoire, Benin and Togo (WHO, 2019).

Buruli ulcer is part of the group of infectious diseases classified in neglected tropical diseases with a poorly known global prevalence and affecting mainly isolated rural African communities. This disease is the third most common mycobacterial infection after tuberculosis and leprosy (OMS, 2010).

According to the WHO, Buruli ulcer begins with a painless nodule, plaque or œdema in the skin which, if left untreated, progresses to massive skin ulceration (OMS, 2012). Currently, only the specific antibiotic treatment, possibly associated or not with the surgery has a proven effectiveness. However, it can only be achieved in medical centers of affected countries with adequate technical facilities and specialized staff (OMS, 2012).

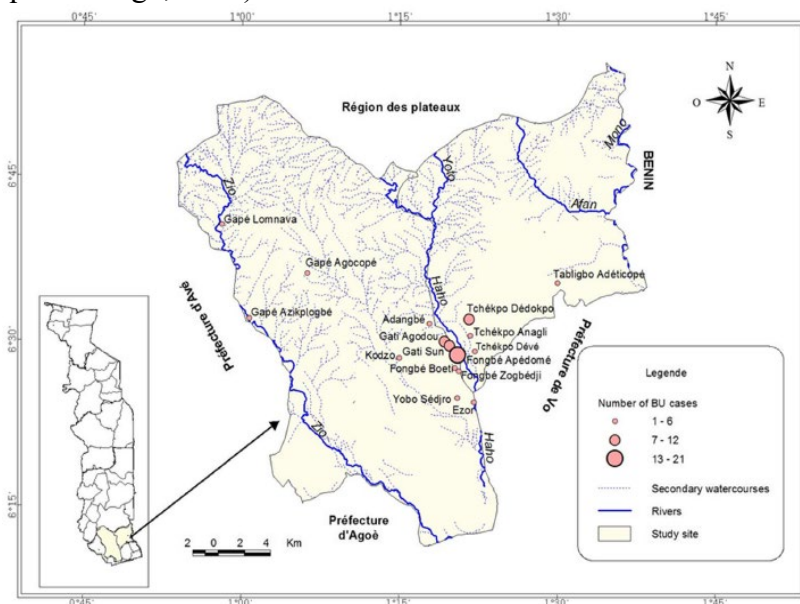
In Togo, the National Buruli Ulcer Control Program have confirmed more than 100 cases from 1996 to 2004. It was advised to patients in endemic areas for early detection of cases and immediate recourse to treatment centers as soon as possible at the first signs of the disease (Bretzel *et al*, 2011). The drug and surgical treatments are offered free of charge to patients, but there is the problem of side effects which can cause sequelae in children including liver, kidney, auditory and vestibular lesions. This treatment also seems to provide various mutilations, even amputations. Moreover, the surgical treatment, in combination or not with the drug treatment, presents satisfactory results, but also other constraints among which the long duration of hospitalizations and of treatment for many patients, mostly from rural areas (OMS, 2012; Hounghédji *et al*, 2011; Zhang *et al*, 2013). As in many African countries, patients prefer to start treatment with traditional medicines to any other form of care because of the lack of information on the cost of hospital care. The use of traditional medicines recipes in the treatment of Buruli ulcer is reported by several study in central and west Africa (Adjet *et al*, 2016; Bayaga *et al*, 2017; Johnson *et al*, 2004; Tsouh *et al*, 2015; Yemoa *et al*, 2008; Yemoa *et al*, 2015). The patients also resort to traditional treatment for cultural reasons because of the tradition which often requires consulting the traditional healer to know the cause of a disease. This treatment of Buruli ulcer is therefore the first resort available to these patients who are generally poor (Johnson *et al*, 2004). Despite the prevalence of this pathology in Togo, few works were conducted on the study of plants used traditionally and in first recourse by the populations of the maritime region, the main endemic center of this pathology in Togo. In order to explore and increase the potential in medicinal plants traditionally used against this pathology, the present study is proposed to identify the plants species and the recipes used by traditional healers in the treatment of BU in Maritime region of Togo.

## **Methods**

### **Study area and population**

This survey was conducted in the maritime region of Togo specifically in 17 villages in Zio and Yoto districts where a recent study focused on the risk factors for *Mycobacterium ulcerans* infection (Maman *et al*, 2018). These districts are in southern Togo (6°30'0" N and 1°19'60" E). With an estimated population of 1,762,518 in 2012, this region most burdens the disease with

more than 85% of confirmed BU patients. The climate is tropical and humid with two rainy seasons and two dry seasons. Our survey was conducted among 16 traditional healers who treat Buruli ulcer. They were recruited from the list provided by the local coordinator of the traditional healers. This region has a good biological diversity in plant species (Rapport national sur la diversité biologique du Togo, 2014).



**Figure 1:** Maritime Region Map presenting villages surveyed, distribution of BU cases and hydrographic network (Maman *et al*, 2018)

## Study method

This study is descriptive and qualitative. The information was collected from traditional healers based on direct observation and interviews. For this the technique of the individual interview with open questions is used. At a first contact, an appointment is requested in view of their availability for the interview. During the interview, data were collected on the diagnostic of the disease, the ingredients of the phytomedicines, their methods of preparations, their galenic form of presentation, the route of administration and the prohibitions during the treatment received by patients.

## Collect and identification of plants

Some plants were immediately available in the habitat of traditional healers and were collected, for the others the collect was done with the help of the traditional healer using the local names. This identification was done with the approval of an expert in traditional medicine based on observation and the local names. Botanical identification continued at the herbarium of the Faculty of Science at the University of Lomé using the leaves and fruits of the various

plants that we collected in the field and the local names. The botanist made the identification based on the specific characteristic of each species using Togo flora and the APG IV nomenclature.

**Data analysis**

The information collected from the survey is entered into the Microsoft Excel 2013 software and analyzed to make the calculations of the Rate of Use (RU) of the plants species. The calculation of the RU of each plant is made according to the study conducted by Yemoa in Benin (Yemoa *et al*, 2008):

$$RU = \frac{\text{Number of users of a given plant}}{\text{Total number of users}} \times 100$$

High utilization rates were those between 40 and 60%, averages between 15 and 39% and low values below 15%.

**Results**

**Sociodemographic data of traditional healers**

This study enrolled 16 traditional healers from the Togo Maritime Region, 10 of which were male and 6 females. Their average age was 42.31 ± 4.24 years with a minimum of 29 years and a maximum of 55 years. Table 1 summarizes the socio-demographic data of these traditional healers. Traditional healers have been divided into four age groups. The analysis of Table 2 shows that the majority of traditional healers was in the two age groups of 30 to 40 and 40 to 50 years of age. These two age groups represent more than 75% of respondents. About 50% of these traditional healers has reached primary school, 6.25% have reached secondary level and 6.25% has reached university level. About the origin of their knowledge, 75% were introduced to the practice of traditional medicine within the family, while the others were initiated outside the family. The results of the survey also revealed that 56.25% of traditional healers practice traditional medicine in full time, while the others practice it as a secondary activity. Concerning the season and the time of harvesting plant organs, some traditional healer prefer the dry season and others the rainy season, but the factor that plays the most is the availability of plants for seasonal plants (Table 1).

Table 1: Sociodemographic data of traditional healers

Sex		Male		Female	
N		10		6	
%		62.5		37.5	
Age ranges	< 30 years	30-40 years	40-50 years	>50years	
N	01	06	07	02	
%	6.25	37.5	43.75	12.5	1,83
Schooling	Illiterate	Primary school	Secondary school	Baccalaureate	
N	06	08	01	01	

%	37.5	50	6.25	6.25
<b>Religion</b>	<b>Animist</b>	<b>Christian</b>	<b>Muslim</b>	<b>others</b>
N	7	5	1	3
%	43.75	31.25	6.25	18.75
<b>Origins of Knowledge</b>	<b>Exclusive Family Heritage</b>	<b>Traditional Initiation</b>		
N	12	4		
%	75	25		
<b>Status of the healer</b>	<b>Full time</b>	<b>Other activity</b>		
N	9	7		
%	56.25	43.75		
<b>Season of picking</b>	<b>Dry</b>		<b>Rainy</b>	<b>All seasons</b>
N	3		7	6
%	18.75		43.75	37.5
<b>Time of day</b>	<b>Morning</b>	<b>Midday</b>	<b>Evening</b>	<b>The whole day</b>
N	9	0	2	5
%	56.25	0	12.5	31.25
The data in the table are expressed in number (N) and percentage (%)				

### Botanical characteristics and diversity of anti buruli plants

In total, 27 species of plants belonging to 20 families have been identified in the treatment of Buruli ulcer in the maritime region of Togo. The most represented families were: Caesalpinaceae with 3 species, Euphorbiaceae, Apocynaceae, Asteraceae Sapindaceae and Annonaceae with 2 species each. The species with the highest frequency of use are *Blighia sapida* KD Koenig (56.25%), *Amaranthus spinosus* L. (50.00%), *Ocimum canum* Sims (50.00%), *Piliostigma thonningii* (Schum.) Milne-Redh (50.00%), *Aloe vera* L. (43.75%), *Anacardium occidentale* L (43.75%), *Paullinia pinnata* L. (43.75%) and *Xylopiya aethiopica* (Dunal) A.Rich (43.75%) (Table2). The plant parts used to make herbal preparations are dry and fresh barks; dry and fresh leaves; roots, stems, fruits, and seeds of fruits. The Leaves were the most used part of plants (48%) followed by roots (21%), then barks (10%), fruits (7%), stems (9%), flowers (5%). These data are presented in Figure. 2, 3.

### Presentation form of phytomedicines and route of administration

The preparation of the medicines employed several methods: decoction (37%), followed by black powdered ointment (24%), maceration (15%), infusion (12%), spraying (6%) and poultices (6%) (Figure 4). The local administration was the predominant route of administration followed by oral administrations. Decoction, infusion, or maceration were reported as taken orally or used to wash or disinfect the wound while black powders ointment, spray, were applied on the ulcer as bandages. During the use of these recipes,

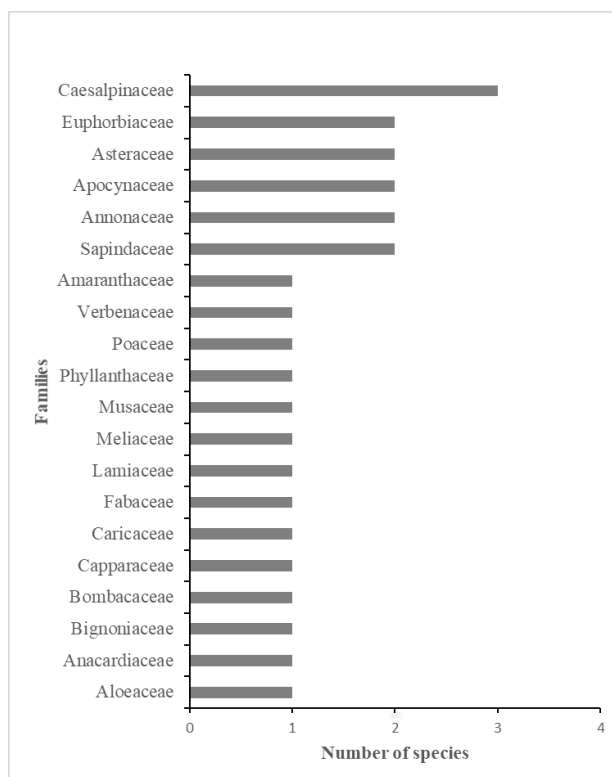
the patients have to avoid the consumption of sugar, red oil, alcohol, and cassava. The global data of the survey is presented in Table 2.

**Table 2:** Plants used in the treatment of Buruli ulcer

Families	Species	Codes	Local Names	Used parts	Galenic Forms	RU (%)
Aloeaceae	<i>Aloe vera</i> L.	-	Aloes	Leaves	decoction and black powder	43.75
Anacardiaceae	<i>Anacardium occidentale</i> L.	TOGO15582	Atchanti	Bark, leaves	decoction and black powder	43.75
Annonaceae	<i>Xylopiya aethiopica</i> (Dunal) A.Rich	TOGO15583	Etso	Fruits	Decoction, infusion	43.75
	<i>Citrus limon</i> (L.) Burm.	TOGO15584	Donti	Fruits	decoction and maceration	37.50
Apocynaceae	<i>Strophantus sarmentosus</i> DC	TOGO15586	Amagan	Leaves, roots	decoction, black powder, poultice	37.50
	<i>Strophanthus hispidus</i> DC	TOGO15372	Aho	Leaves, roots	decoction and maceration	37.50
Asteraceae	<i>Acanthospermum hispidum</i> D.C.	TOGO15585	Ahunglunvi	Leaves	decoction, black powder	37.50
	<i>Ageratum conyzoides</i> L.	TOGO15587	Agbadjadjigbé	Leaves, roots	Spray, infusion and maceration	25.00
Bignoniaceae	<i>Kigelia africana</i> (Lam.) Benth.	TOGO15590	Gnakpekpe	Leaves	spray, maceration, decoction	31.25
Bombacaceae	<i>Adansonia digitata</i> L.	TOGO15588	Adidoti	Bark	Decoction, black powder Maceration	37.50
Caesalpinaceae	<i>Erythrophleum suaveolens</i> Guill. & Perr.) Brenan	-	Atiuhia	Bark, roots	Spray ,maceration, decoction, infusion	25.00
	<i>Caesalpinia bonduc</i> L. Roxb	TOGO15589	Adikou	Leaves, fruit, roots	decoction, maceration	25.00
	<i>Cassia alata</i> L. Roxb	TOGO15591	Patapa	Leaves, roots	decoction ,black powder, poultice infusion	37.50
Capparaceae	<i>Crateva religiosa</i> G. Forst	TOGO15592	Awantagnizan	Leaves, roots	Decoction, black powder	18.75
Caricaceae	<i>Carica papaya</i> L.	TOGO15604	Ahubati	Roots, leaves	Black powder, decoction	37.50
Euphorbiaceae	<i>Euphorbia hirta</i> (L.) Millsp	TOGO15593	Anonsika	Leaves	Decoction, black powder	37.50
	<i>Alchornea cordifolia</i> (L.) Millsp	TOGO15594	Avlovlo	Leaves	Decoction, infusion spray, maceration	18.75
Fabaceae	<i>Piliostigma thonningii</i> (Schum.) Milne-Redh	TOGO15595	Eklo	Leaves, stems	Decoction ,black powder, infusion	50.00
Lamiaceae	<i>Ocimum canum</i> Sims	TOGO15596	Ahamè	Leaves, stems, Flowers	Decoction, black powder	50.00

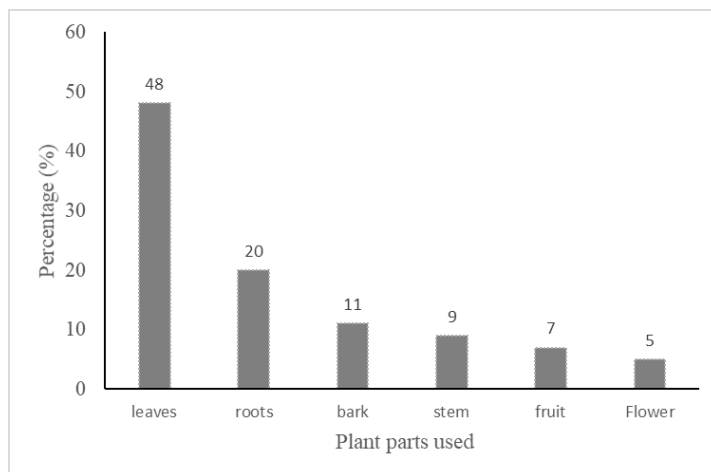
Meliaceae	<i>Azadirachta indica</i> A.Juss	TOGO15597	Kiniti	Leaves, roots ,bark	Decoction, infusion black powder,	31.25
Musaceae	<i>Musa paradisiaca</i> L.	-	Amadanti	Trunk	Decoction, maceration	31.25
Phyllanthaceae	<i>Phyllanthus amarus</i> L.	TOGO15598	Ehliivi	Leaves, stems	Decoction, black powder poultice	37.50
Poaceae	<i>Zea Mays</i> L.	TOGO15599	Ebli	Flowers	Decoction, black powder	12.50
Sapindaceae	<i>Paullinia pinnata</i> L.	TOGO15600	Asiasion	Leaves	Decoction, black powder Maceration, infusion	43.75
	<i>Blighia sapida</i> K. D. Koenig	TOGO15601	Atchanti	Bark, leaves	Decoction, Maceration, Poultice	56.25
Verbenaceae	<i>Tectona grandis</i> L.f	TOGO 15602	Tecti	Leaves	Decoction, black powder	6.25
Amaranthaceae	<i>Amaranthus spinosus</i> L.	TOGO15603	Aklantigbé	Leaves	decoction, poultice	50.00

RU: Rate of use

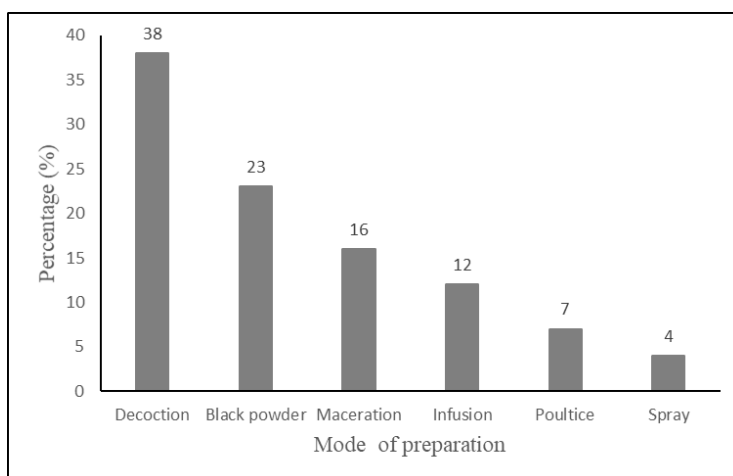


**Figure 2:** Anti buruli ulcer plant species distribution among families in Togo Maritime Region





**Figure 3:** Frequency of plants part used



**Figure 4:** Repartition of forms of herbal preparations

## Discussion

The present study was interested to the identification and ethnobotanical study of plants used in the traditional treatment of buruli ulcer in the maritime region of Togo. Our first observation is that traditional healers prospected were mostly male seniors. This profile of traditional healers in the Maritime Region of Togo is observed in most studies of this kind, confirming that the practice of traditional medicine is reserved for mature men (Aburjai *et al* 2007). The majority of people respondents were over 42 years of age. Indeed older people are expected to provide more reliable information, because they hold much of the ancestral knowledge (Lakouéténé *et al*, 2009).

From the plants they use, 27 species have been identified. They are grouped into 20 families among which the more represented in species are Caesalpinaceae, Euphorbiaceae, Annonaceae, Apocynaceae and Asteraceae. The Euphobiaceae, Apocynaceae, Annonaceae families are also represented in studies conducted in tree West African countries (Tsouh *et al*, 2015). In 2016, Adjet *et al* conduct the same study in the Yamoussoukro health district and identified 15 plant species, four of which are found in our study: *X. aethiopica*, *Musa paradisiaca* L., *P. thonningii*. and *C. papaya*. In Benin, a similar study conducted in 2008 by Yemoah *et al* in Ouinhi commune, identified 49 plant species, five of which are found in our study: *X. aethiopica*, *Strophanthus hispidus* DC, *Erythrophleum suaveolens* Guill. & Perr. Brenan, *P. thonningii*, *P. pinnata*. Among the most cited species in our study, previous work has demonstrated from various extracts the presence of molecules with biological properties in wounds healing. This is the case of *A. vera* which is a perennial plant widely used for the treatment of various diseases, including Buruli ulcer and extracts of its leaves showed a MIC of 40 µg/ml on *M. ulcerans* (Addo *et al*, 2007). Many of its chemicals have been identified such as carbohydrate polymers; glucomannans, phenolic compounds such as chromone and anthraquinone. Naturally, the gel is used for its wound healing potential because its high water content can keep the wound moist and promote the migration of epithelial cells. In addition, it has been suggested that the main carbohydrate, mannose-6-phosphate, plays an important role in the healing potential of wounds (Davis *et al*, 1994). *Ocimum canum* Sims contains rosmarinic acid which is known for its anti-inflammatory, antibacterial properties and the extracts of this plant are widely used in traditional pharmacopoeia (Tshilanda *et al*, 2016). The different parts of the western *Anacardium* species are known for their antimicrobial properties (Maia *et al*, 2000; Kubo *et al*, 2003; Gonçalves *et al*, 2012; Akinjogunla *et al*, 2012; Saidu *et al*, 2012; Marques *et al*, 1992; Laurens *et al*, 1982). The evaluation of the antimicrobial activity of ethanolic extracts of flowers, leaves, bark showed minimal bactericidal and fungicidal activities against: *Streptococcus mutans*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, MRSA, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Helicobacter pylori*, *Candida albicans* and *Candida tropicalis*. An antioxidant activity was also observed on the extracts (Silva *et al*, 2016). *X. aethiopica* is a plant that contains many bioactive compounds with antioxidant, anti-inflammatory, antimicrobial and anti-cancer properties. Fifty-eight bioactive compounds were identified from the methanol extract of *X. aethiopica* fruits including: 2,4,6-octatriene (2.74%), cyclohexanemethanol (2.57%), kaurene (3.59%), 9, 12-octadecadienoic acid (5.63%), 1,6-cyclodecadiene (10.81%), terpineol (3.22%), cyclohexene (3.32%), copaene (3.04%), 1,6-

cyclodecadiene (5.53%), 1-hexadecyne (5.63%) and silane (4.63%) (Okagu *et al*, 2018). *S. hispidus* is also used in the traditional treatment of infectious diseases. Antimicrobial tests from the crude extracts of parts of the plant have shown that these extracts inhibit most of the organisms tested: *S. aureus* NCTC 6571, *Bacillus subtilis*, *E. coli* NCTC 9001, *P. aeruginosa* NCTC 6570, *Aspergillus niger* and *Candida* (Idowu *et al*, 2005). *P. thonningii* is a plant widely used in traditional pharmacopoeia with antibacterial, antifungal, antimalarial, anti-helmintic properties, and methanolic extracts of stem bark exhibited activity against bacterial isolates at a concentration of 20 mg/ml (Akinpelu *et al*, 2000; Madara *et al*, 2010; Kwaji *et al*, 2010; Asuzu *et al*, 1991; Agban *et al*, 2013). *P. pinnata* is widely used in cosmetology to treat fungal infections and various skin infections (Batawila *et al*, 2007). *P. amarus* is also known to have antibacterial, antimycobacterial properties (Bakary *et al*, 2011; Oluwafemi *et al*, 2010; Zubair *et al*, 2017; Babatunde *et al*, 2014). The different parts of these plants are used in various forms including decoction and maceration as in other studies conducted in Ghana, Ivory Coast, Benin, Cameroon and the parts of the plants used are made up of leaves, barks and roots (Adjet *et al*, 2016; Bayaga *et al* 2017; Tsouh *et al*, 2015). In our study, the method of carbonization and reduction in black powder of parts of selected plants has been widely used by traditional healers. This practice is also adopted in Benin (Yemoa *et al*, 2008). In Togo, the diagnosis of the disease is made by the difficulty of wound healing and the healers begin the treatment by considering its spiritual dimension and carry out the exorcism by sacrifices to the spirits of the ancestors to promote an effective cure. The treatment continue by the disinfection and wound treatment by herbal remedies. Johnson *et al* (Johnson *et al*, 2004) have found in Benin four stages of treatment with a traditional healer, including diagnosis of the disease, removal of necrotic tissue, wound care and exorcism.

Most of the recipes collected in the maritime region consisted of at least two plant species. Several studies of traditional treatments for Buruli ulcer in Africa have highlighted this complexity in the preparation and content of remedies, which rarely include a single plant species (Bayaga *et al*, 2017). This form of association of various plant species in treatments or recipes could present risks of interaction or toxicity (Yemoa *et al*, 2008). Several studies have shown positive interactions of these mixtures in inhibiting the *in vitro* growth of *M.ulcerans*. A mixture of *Zea mays* L. and *Spigelia anthelmia* L. has exhibited a MIC of various concentrations of the extract ranging from 6.25 to 25 µg / mL (Addo *et al*, 2007). *Z. mays* is known for it wound healing activity (Sravania *et al*, 2012). Corn silk is rich in phenolic compounds, especially flavonoids. It also consists of proteins, vitamins, carbohydrates, calcium, potassium, magnesium and sodium salts, volatile oils and steroids such as sitosterol and stigmasterol, alkaloids and saponins (Hasanudin *et al*

2012) which could promote healing and the antibacterial activities of the mixture. A mixture of *X. aetiopica* and *C. papaya* is also used in Côte d'Ivoire (Adjet *et al*, 2016) to treat Buruli ulcer. These mixtures offer the advantage of part of their phytochemical composition to promote good healing. *Musa paradisiaca* is also endowed with a good antimycobacterial activity with a beneficial interaction with several other plant species. Bayaga *et al* found in Cameroon that the plant was associated with 18 herbal recipes used in the fight against Buruli ulcer (Bayaga *et al*, 2017). Toxicity studies on most medicinal plants and interactions using animal models have provided results that strengthen their use among humans; however, many such plants could be associated with some toxicity (Nasri *et al* 2013, Yemoa *et al*, 2008). This is the case of *Carica papaya* extracts. The tests on normal human liver cells of its extracts showed a cytotoxicity of 50%. (Tsouh *et al*, 2016). Os *et al* in 2019 have shown that hydroethanolic extracts from leaves of *O. canum* have subacute toxicity in rats treated by oral route (Os *et al*, 2019). However, some authors emphasize that the actual therapeutic activities of certain medicinal plants cannot be explained by the mere presence of one or other of the constituents. In these mixtures based on extracts of various plants, it is the association or synergy that exists between the constituents, which is very often responsible for the desired effect (Fleurentin *et al*, 2011). This ethnopharmacological reasoning, which holds a preponderant place in the search for new treatments of tomorrow, puts therefore more and more emphasis on mixtures.

There is therefore a close relationship between these plants and the fight against ulcers in different African cultures. Their use in different cultures attests it. They could have properties and therapeutic potentials related to ulcerations that start with a nodule. The idea of the prohibition accompanies the care in the context of African traditional medicine and in our study the forbidden foods to the patients of Buruli ulcer are identical to those forbidden to the patients in Ivory Coast by the traditional healers (Adjet *et al*, 2016).

## Conclusion

The treatment of Buruli ulcer uses specific antibiotics that sometimes come up against relapses and therapeutic failures and sometimes the side effects felt by the patients leave sequelae. Thus, other ways of research such as the use of herbal medicine should be explored to improve the management of the disease. Our study has allowed us to identify twenty-six plants, eight of which (*B. sapida*, *A. spinosus*, *O. canum*, *P. thonningii*, *A. verra*, *A. occidentale*, *P. pinnata* and *X. aethiopica*) have a very high Rate of Use. Through the current stage of the results of our study, a collaboration between traditional medicine and modern medicine should be considered for research

in therapeutic innovation by ethnopharmacological reasoning for chemical and pharmacological evaluation to identify new molecules to treat Buruli ulcer.

### **Competing interests**

Authors declared they have no conflict of interest.

### **Authors' contributions**

All the authors contributed to the conduct of this work. The authors of this article have read and approved its content.

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