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## Research Article

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## Plants used in Bandjoun village (La'Djo) to cure infectious diseases: An ethnopharmacology survey and *in-vitro* Time-Kill Assessment of some of them against *Escherichia coli*

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### ABSTRACT

An ethnopharmacology survey concerning the medicinal plants used in Bandjoun village (La'Djo) to cure infectious diseases was carried out in three districts of this village. The survey led to the identification of 79 medicinal plants species listed in 41 families. These plants were cited to be used to treat about 25 infectious diseases among which malaria, diarrhea and intestinal-worms were the most cited. *Chromolaena odorata*, *Voacanga africana*, *Moringa oleifera*, *Mammea africana*, *Euphorbia hirta*, *Psidium guajava*, *Allium cepa*, *Enantia chlorantha*, *Alstonia boonei* and *Picralima nitida*, were the ten most cited plants. Extractions of parts of these last plants were performed in hydro-ethanol (3:7) solvent and then tested *in-vitro* against an *Escherichia coli* isolate. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were assessed by microdilution assay and the time-kill assessment was carried out by measure of log reduction in viable cell count, on a period of 48 hours. MIC and MBC determined were ranged between 1.00 and 32.0 mg/mL. Eighty percent (80%) of plant extracts tested have been bactericidal (MBC/MIC = 1 or 2) after 24 hours of incubation. A significant dose-dependent decreasing ( $P < 0.05$ ) in test organisms population was observed in the time with log reduction in viable cell count was ranged between 0.13 log<sub>10</sub>cfu/mL and 100% of inhibition. This antimicrobial activity has been attributed to metabolites groups in plant extracts namely, Phenols, flavonoids, tannins, coumarins, terpenoids, anthraquinones, cardiac glycosides, anthocyanides and alkaloids. These results obtained against *Escherichia coli* give a scientific validation to the traditional medical knowledge of Bandjoun-village populations and confirm some of the plants identified like a source of potentially active compounds against infectious diseases.

**Keywords:** Infectious diseases, Medicinal plants, Ethnopharmacology survey, Bandjoun village (La'Djo).

### INTRODUCTION

In Cameroon, infectious diseases are amongst the most commonly notified diseases and largest cause of mortality<sup>1</sup>. The major infectious diseases associated with a high degree of risk within the population include food or waterborne diseases (bacterial and protozoal diarrhea, hepatitis A and E, and typhoid fever), vector borne diseases (malaria and yellow fever), water contact disease (schistosomiasis), respiratory disease (meningococcal meningitis), and animal contact disease (rabies)<sup>2,1</sup>. Infections like malaria, diarrhea, fungal infections, HIV/AIDS, tuberculosis, scabies, measles and acute infections of the respiratory tracts, are becoming more prevalent. Among human pathogenic bacteria, *Escherichia coli* are Gram negative bacteria known to be implicating in most clinical case. In Cameroon, these bacteria are cited in many health centers reports to be associated to chronic diarrhea in infants, hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura in adults. Transmitted by the fecal or oral route, infectious dues to *Escherichia coli* strains are among the major causes of morbidity and mortality. The emergency and propagation of the microbial resistance and the toxicity problem of some effective drugs now available have been reported in many health investigations. There is the time to search about new therapeutic drugs.

Medicinal plants are sources of important quantities of chemical substances which are able to initiate different biological activities including those useful in the treatment of human diseases<sup>3</sup>. History shows that plants have been an important source of medicines against microbial infections. Today, the values of medicinal plants as starting point for discover of new therapeutic compounds are well-known. Interest carried to medicinal plants like abundant source of bioactive compounds has not stopped to increase. Many plants species are traditionally known for their anti-infectious properties and few of them have been subjected to scientific studies concerning their active principles or their utilization like complementary medicines in modern therapy. Scientific investigation of medicinal plants used in folklore remedies have attracted increased attention in the world of medicine, especially in a bid to finding lasting solutions to the problems multiple resistance to the existing conventional antimicrobials<sup>4</sup>. The researchers of new compounds with anti-infectious virtues stay a great challenge through the world

and in particularly in Africa where the prevalence of infectious diseases stays very alarming regarding the mortality and morbidity levels.

Cameroon is a country of central Africa administratively divided in ten (10) regions among which West-Cameroon region. This region of Cameroon is sociologically represented by three principal ethnical groups namely, Bamiléké (or Grassfields), Bamoun and Tikar. These ethnical groups are closed in 127 traditional kingdoms among which Bandjoun village constitutes one of the most important. Geographically, bordering to others rural communities (Bafang, Dschang, Ngiemboon, Ngomba, Bali, Bamoun and Bangou), Bandjoun village is a transit zone inhabited by a cosmopolite population. The heterogeneity of the population, regrouping native population and exogenous population come from others ethnical groups, has permit for a long time the exchange of knowledge concerning the medicinal practices. Further, native populations in this village are known to be very attached to her socio-cultural values. They still possess and preserve their traditional patrimony. The knowledge about the therapeutics values of medicinal plants remains transmitted the generation to generation and the information stay conserved in the time. In Bandjoun village, medicinal plants constitute a precious patrimony and many plants species are frequently used to ensure the primary medical care. According to her geographic location and to the predominance of phytomedicine like main way to cure illness, this village is a tank of medicinal practices and medication. Therefore, this study was undertaken to catalogue, identify and promote anti-infectious medicinal plants used in this village.

## MATERIAL AND METHODS

### Study Area

This study was carried out in Bandjoun village. Vernacularly called La'Djo in the local language (Ghomala'), this village of 274 km<sup>2</sup> is situated in west-Cameroon region (5° 22' 31" Nord, 10° 24' 44" Est, 1 515 m of altitude). The cosmopolite living population is estimated at 70 000 peoples, installed in 7 provinces called "Jie" which are traditional administrative units (Jie Djiomghuo, Jie-Se, Jie-Leng, Jie-Theghem, Jie-Kouo', Jie-Sè and Jie-MBem). Bandjoun village is crossed by a climate of tropical Sudanese type, characterized by a dry season which runs from October-November to March-April and a rainy season that starts in March-April and lasts until October-November. Temperatures range between 15°C and 30°C in average with high daily variation. The average temperature is 25°C. The terrain is mountainous and the vegetation is characterized by forests with large number of trees and leafy shrubs throughout the year. Agriculture is enough practice in this village and a diversity of economic plants is cultivated, including medicinal plants.

### Methodology of survey

The ethnopharmacological survey was carried out from the 04<sup>th</sup> to the 15<sup>th</sup> January 2015. This study was conducted in 03 districts of Bandjoun-village namely Kamgo, Pête and Famleng. A total of 46 dwellers were submitted to an semi-structured interview concerning her general knowledge on plant species used as a remedy to cure microbial infections, the parts of plant used (e.g. barks, leaves, roots or fruits), mono-specific preparation of the recipes (e.g. decoctions or infusions) and the mode of administration of the recipes. Among the interviewed dwellers there had 26 healers, 05 herbalists and 15 sellers of medicinal plants. They were equally questioned because of their equal access to natural medicines. The study was conducted by 02 PhD students of Departments of Biochemistry of Yaoundé I and by 07 villagers living in Bandjoun village. All the investigators spoke local language (Ghomala'). The provided information was collected using a questionnaire. To insure viability and authenticity of gathered information, the old persons have preferably interviewed (46 years old more). The survey was conducted according the principles laid out by the Nagoya protocol. The Fidelity level *FL* (%) which is a percentage

of informants concerning a use of each plant species to cure infectious disease was determined as the following:

$$FL (\%) = \frac{\text{Number of citations of each plant}}{\text{Total number of citations}} \times 100$$

### Identification of species

The plant species indicated was immediately identified by different investigators in the inquiry areas concerning their local names. Some of these plants were collected with the agreement of the villagers and the further identification concerning theirs scientific names and theirs botanical families was performed at national herbarium of Cameroon. Some plant species was identified at laboratory of phytochemistry of University of Yaoundé I. After identification, the scientific names were confirmed using the net-work to check that the given scientific name corresponded effectively to the plant species identified.

### Relative predomination in infectious diseases cited

The relative predomination in infectious diseases *RPD* (%) cited was evaluated based on the number of citations made by indigenous peoples interviewed for each specific disease as the following:

$$RPD (\%) = \frac{\text{Number of citations of the disease}}{\text{Total number of citations}} \times 100$$

### Variability in preparation modes of the recipes

During the survey the data concerning the mono-specific preparation methods of the recipes were collected. In terms of the number of citations, the percentage of traditional methods of preparation *PM* (%) of different plant recipes was evaluated as the following:

$$PM (\%) = \frac{\text{Number of citations of each preparation mode of the recipes}}{\text{Total number of citations}} \times 100$$

### Variability in plant parts used

For the preparation of recipes, many plant parts have been mentioned during the survey. The use frequency of any plant part *UF* (%) was determined using the formula:

$$UF (\%) = \frac{\text{Number of citations of each plant part}}{\text{Total number of citations}} \times 100$$

### Variability in administration modes

The proportion of any administration mode *PAM* (%) of plant recipes was determined according of number of citations as the following:

$$PAM (\%) = \frac{\text{Number of citations of each administration mode}}{\text{Total number of citations}} \times 100$$

### Methodology for extraction and time-kill analysis

#### Plant material

According to the fidelities levels *FL* (%) registered during the ethnopharmacological survey, some plant parts of the most cited plant species were collected and tested *in vitro* against *Escherichia coli* isolate. These plant parts consisted to the stem barks of *Voacanga africana*, *Moringa oleifera*, *Picalima nitida*, *Alstonia boonei*, leaves of *Euphorbia hirta*, *Psidium guajava*, *Chromolaena odorata*, *Mammea africana*, *Enantia chlorantha*, and bulb of *Allium cepa*. The harvest has been done with the approbation of villagers. Notably that some plant parts (stem barks of *Picalima nitida* and *Alstonia boonei*)

were bought to the market during the interview with medicinal plants sellers.

### Plant extracts

The plant parts (stem bark or leaves) were collected separately, cleaned, air-dried at room temperature and crushed using electric grinder. A weighed quantity of 100 g of any powder was extracted in 500 mL of hydro-ethanol mixture (3:7). After 72 hours of maceration, any extract was filtered through Watman No 1 filter paper and concentrated under reduced pressure using a rotary evaporator (Laborota 4000-efficient, Heldolph, Germany). Extractions were repeated three times. Eighteen milligrams (160 mg) of each extract were weighed and dissolved in 5ml of sterile Mueller Hinton Broth medium (MHB) (Sigma, St Louis, USA) to give a stock solution at 48 mg/mL used for the biological tests. The extraction yield *EY* (%) was determined according the following formula:

$$EY (\%) = \frac{\text{Mass of extract obtained}}{\text{Mass of powder extracted}} \times 100$$

### Phytochemical screening

Qualitative phytochemical tests were done according the standard procedures described into literature by Trease and Evans<sup>5</sup>, Sofowora<sup>6</sup>, Harbone<sup>7</sup> and Edeoga *et al.*<sup>8</sup>.

### Determination of minimum inhibitory concentration (MIC)

Susceptibility testing of *Escherichia coli* to vegetal extracts was performed by measuring MIC following the CLSI M27-A2 guidelines<sup>9</sup>. A volume of 200  $\mu$ L of vegetal extract stock solution (48 mg/mL) was introduced into the first row of the microtiter plate. To all other wells 100  $\mu$ L of double strength MHB was added. Then, double dilutions of tested substances were performed. The range of final concentrations tested were 0.25 to 32.0 mg/mL for each vegetal extract and 0.03 to 4.00 mg/mL for Gentamicin (Brunhild Pharmaceutical Private Limited), included as positive antibacterial control. From a bacterial culture of 24 hours on Mueller Hinton agar (MHA) plate, the inoculum was prepared in sterile MHB at 0.5 McFarland. An aliquot of 50  $\mu$ L of bacterial inoculums was added to each well of plate. The controls for the bacterial growth and the medium sterility were realized. After 24 hours of incubation at 35°C, the MIC was determined by addition of 50  $\mu$ L of 2,3,5-triphenyl tetrazolium chloride (TTC, Sigma-Aldrich) at 2.0 % into each well of the microtiter plate and the plates were incubated for 1 h at 35°C. Bacterial growth was assessed by a reddish-pink color and the MIC was determined as the lowest inhibitory concentration of plant extract for which the absence of visual color change was observed after the addition of TTC. Each assay was performed in triplicate.

### Determination of minimum bactericidal concentration (MBC) and bactericidal action

MBC were determined by subcultures on agar plate. An aliquot of 100 $\mu$ L of each of the wells with concentration greater than or equal to MIC was spread onto MHA. The MHA plating were incubated at 35°C for 24 hours and MBC was considered as the lowest concentration where there was no resumption of bacteria growth. According to Fauchère and Avril<sup>10</sup> when the MBC/MIC ratio is equal to 1 or 2, the antibiotic is bactericidal and when this ratio is  $4 \leq \text{MBC/MIC} \leq 16$ , the antibiotic is bacteriostatic.

### Time-kill assay

The time-kill of *Escherichia coli* by vegetal extracts was assessed according the modified protocol described by Eliopoulos and Moellering<sup>11</sup>, with some modifications. From a bacterial culture of 24 h on MHA plate, the starting inoculum was prepared in sterile MHB at approximately  $10^6$  cfu/mL using McFarland. One milliliter (1 mL)

of bacteria suspension was added to 9 mL of MHB with vegetal extract to test at 0.5 $\times$ MIC, 1 $\times$ MIC and 2 $\times$ MIC. The preparations were then incubated at 35°C. A growth control containing only the bacterial strain was performed. At time-kill period of 0, 6, 12, 18, 24, 30, 36, 42 and 48 hours, an aliquot of 500  $\mu$ L was removed from each culture and serial tenfold dilutions were prepared in saline (NaCl 0.9%) as needed. The numbers of viable cells were determined by the plate count technique. Hundred microliters (100  $\mu$ L) of each dilution were plating on a MHA plate and the plates were incubated at 35°C for 24 hours. Emergent bacterial colonies were counted and cfu/mL was determined. Kill curves were plotted with time against logarithm of colony forming unit per milliliter (cfu/mL). All experiments were repeated at least three times. Relative to the starting inoculums, the bactericidal activity was defined as reduction in the viable colony count resulting to  $3 \log_{10}$  (cfu/mL).

### Statistical Analysis

The statistical analysis and the diagrams were performed using GraphPad Prism 5 software. Differences between the means were statistically compared by Dunnett's post hoc multiple comparison tests. The values were considered significantly different when  $P < 0.05$ .

## RESULTS

### Ethnopharmacology survey

The survey realized in Bandjoun-village has permit to identify and to collect a total of 79 medicinal plants species listed in 41 families (**Table 1**). Euphorbiaceae was the most used family with 6 species, followed by Apocynaceae, Annonaceae, Fabaceae, Rutaceae with 5 species for each family, respectively. These plants species were mentioned in the treatment of 25 infectious diseases among which malaria (18.93%), diarrhea (16.58%) and intestinal-worms (10.17%) were the most cited (**Table 2**). According to the Fidelity level *FL* (%), the most cited plant species were *Voacanga africana* (4.22%), *Chromolaena odorata* (4.22%), *Moringa oleifera* (3.59%), *Euphorbia hirta* (3.19%) and *Mammea africana* (3.19%). For the preparation of traditional recipes, thirteen (13) plant parts were mentioned to be used. The use proportion of each plant part has been assessed according the number of citations registered during the investigation (**Table 3**). Stem bark (31.92 % of citations), leaves (30.82 %) and roots (14.24 %) were the most-used plant parts. Concerning the preparation modes of the recipes, 07 modes have been catalogued. Their traditional use frequencies are closed in **Table 4**. The most used preparation mode was decoction in water with 53.24% of citations, followed by infusion in water (19.15%) and crushes (10.55%), respectively. These recipes were administered through 3 mains routes which were: Oral (drinks or eats), topical (rub, bath, chew or ointment) or rectal routes. Oral route was the most used with frequency of 70.89%, followed by topical route (28.16%) and rectal route (0.93%), respectively. **Tables 5 and 6** summarize the administration routes inventoried and their citation percentage.

**Table 1:** Medicinal plants species inventoried, ailment treated and mode of preparation and administration of recipes

Families	Plant species	Common names	Vernacular names	Ailment treated	Parts used	Method of preparation of recipes	Mode of administration	NC	FL (%)
Acanthaceae	<i>Brillantasia patula</i> T. Anderson	Lemba lemba	Yoruba ọwọ (Nigeria)	Rheumatism	Stem bark	Decoction in water	Drink/Oral	04	1.40
				Mycosis	Leaves	Crush	Rub/Topical	03	
				Scabies				02	
	<i>Eremomastax speciosa</i> (Hochst.)	Mende wote	Pèkuijum	Typhoid fever	Leaves	Infusion/Decoction in water	Drink/Oral	04	1.09
			Malaria	03					
Anacardiaceae	<i>Anacardium occidentale</i> L.	Cashew Plant	Cajueiro (Portuguese)	Diarrhea	Leaves	Infusion in water	Drink/Oral	04	1.09
					Roots			03	
	<i>Lannea acida</i> A. Rich.	Akye ébruhé	Elang (Oku)	Skin infections	Roots	Infusion in water	Rub/Topical	03	1.09
				Toothache	Stem bark			04	
	<i>Pseudopondias microcarpa</i> (A. Rich.) Engl.	Ochol	Nkangela (Ewondo)	Intestinal-worms	Stem bark	Decoction in water	Drink/Oral	05	0.78
<i>Mangifera indica</i> L.	Mango tree	Tse-Megoum	Malaria	Leaves	Decoction in water	Drink/Oral	05	1.72	
			Diarrhea	Stem bark			06		
Annonaceae	<i>Cleistopholis patens</i> (Benth.) Engl. & Diels	Salt and oil tree	Edo ótù (Nigeria)	Intestinal-worms	Leaves	Infusion in water	Drink/Oral	03	1.09
					Stem bark			04	
	<i>Enantia chlorantha</i> Oliv.	Yellow bark	Lemm	Malaria	Stem bark	Decoction in water	Drink/Oral	11	2.97
				Amoebic dysentery	Stem bark			Infusion in water	
	<i>Anona muricata</i> L.	Soursop tree	Ebom ntangan (Beti)	Amoebic dysentery	Leaves	Infusion/Decoction in water	Drink/Oral	05	1.40
				Intestinal-worms				04	
	<i>Cananga odorata</i> (Lam.) Hook. f. & Thomson	Ylang-ylang tree	Ylang-ylang	Scabies	Flowers	Crush	Rub/Topical	03	1.09
Stem bark					04				
<i>Xylopiya aethiopica</i> A. Rich.	African pepper	Bikui (Beti)	Intestinal-worms	Stem bark	Decoction in water	Drink/Oral	03	1.56	
			Toothache	Roots			-		Chew/Topical
			Wounds disinfection	Seeds	Poultice	Rub/Topical	04		
Apiaceae	<i>Apium graveolens</i> Linn.	Celery	Célerie	Rheumatism	Whole plant	Infusion in water	Drink/Oral	04	0.93
Apocynaceae	<i>Picralima nitida</i> (Stapf) Th & H. Dur.	Quinkeliba	Quinkeliba	Malaria	Stem bark	Decoction in water	Drink/Oral	15	2.34
	<i>Alstonia boonei</i> De Wild.	Quinine bush	Ekuk (Ewondo)	Amoebic dysentery	Leaves	Decoction in water	Drink/Oral	05	2.66
				Malaria	Stem bark			12	
	<i>Catharanthus roseus</i> (L.) G. Don	Rosy periwinkle		Malaria	Leaves	Decoction in water	Drink/Oral	02	0.78
				Intestinal-worms	Roots			03	
	<i>Rauvolfia macrophylla</i> Ruiz & Pav.	Essombo	Essombo	Malaria	Stem bark	Decoction in water	Drink/Oral	05	0.78
	<i>Voacanga africana</i> Stapf ex Scott-Elliot	Voacanga	Obeton (Bulu)	Malaria	Roots	Maceration in water	Drink/Oral	12	4.22
				Intestinal-worms	Leaves			08	
Pediculosis				Roots	Infusion in water	Bath/Topical	03		
Toothache				Latex	Maceration in water	Teeth bath/Topical	04		
Asteraceae	<i>Vernonia amygdalina</i> Del.	Ndolè	Djap	Scabies	Leaves	Crush	Rub/Topical	13	2.03
	<i>Chromolaena odorata</i> (L.) King & H.E. Robins.	Christmas bush	Mrés Paul Biya	Mycosis	Leaves	Crush	Rub/Topical	06	4.22
				Scabies		Poultice	Rub/Topical	05	

				Wounds disinfections				16	
Bignoniaceae	<i>Kigelia africana</i> (Lam.) Benth.	Sausage tree	umFongothi (Zulu)	Diarrhea	Stem bark	Maceration in water	Drink/Oral	04	0.93
				Syphilis	Fruit			02	
Burseraceae	<i>Dacryodes edulis</i> H.J. Lam	Safou	Tse-tsem	Skin infections	Bark resin	Maceration in water	Bath/Topical	02	0.31
Cannabaceae	<i>Trema orientalis</i> (L.) Blume	Charcoal-tree		Amoebic dysentery	Stem bark	Decoction in water	Drink/Oral	03	0.93
				Toothache				Maceration in water	
Caricaceae	<i>Carica papaya</i> L.	Pawpaw tree	Papaye	Malaria	Roots	Infusion/Decoction in water	Drink/Oral	04	2.19
				Diarrhea	Seeds			04	
				Intestinal-worms	Leaves			06	
Caesalpiniaceae	<i>Erythrophleum guineense</i> G. Don	Forest ordeal tree	Sassy bark	Diarrhea	Leaves	Decoction in water	Drink/Oral	03	0.46
Cesalpiniaceae	<i>Guibourtia tessmannii</i> (Harms)	Bubinga	Bubinga	Malaria	Stem bark	Decoction in water	Drink/Oral	07	1.56
				Diarrhea				03	
	<i>Cassia alata</i> (L.) Roxb.	emperor's candlesticks	Ngom ntangan (Ewondo)	Dermatitis	Leaves	Crush	Rub/Topical	03	1.25
				Malaria	Roots	Decoction in water	Drink/Oral	05	
Chenopodiaceae	<i>Chenopodium ambrosioides</i> L.	Mexican tea	Elo'o nson (Bulu)	Dermatitis	Leaves	Crush	Rub/Topical	05	1.09
				Intestinal-worms	Aerial part	Infusion in water	Rectal route	02	
Clusiaceae	<i>Mammea africana</i> Sabine	African Mammy Apple	Abodzok (Ewondo)	Vaginal infections	Stem bark	Decoction in water	Bath/Topical	08	3.12
				Syphilis				04	
				Rheumatism	Fruit			Maceration in water	
Combretaceae	<i>Terminalia superba</i> Engl. & Diels	Limba	Korina	Viral infections	Stem bark	Decoction in water	Drink/Oral	04	2.03
				Intestinal-worms				09	
Costaceae	<i>Costus phyllocephalus</i> K. Schum			Measles	Leaves	Poultice	Rub/Topical	02	1.09
				Tuberculosis	Roots	Decoction in water	Drink/Oral	03	
				Rheumatism				02	
Crassulaceae	<i>Kalanchoe crenata</i> (Andrews) Haw.	Neverdie	Ntengueyou (Bayangam)	Cough	Leaves	Decoction in water	Drink/Oral	05	0.78
Cucurbitaceae	<i>Momordica charantia</i> L.	Bitter melon	Pouok	Hepatitis	Leaves	Infusion in water	Drink/Oral	04	0.62
Dilleniaceae	<i>Tetracera pottatoria</i> Afzel. ex G. Don	-		Amoebic dysentery	Stem bark	Maceration in palm wine	Drink/Oral	03	0.46
Euphorbiaceae	<i>Alchornea cordifolia</i> (Shumach.) Müll. Arg.	Christmas bush	Aboué (Ewondo)	Gastric ulcers	Stem bark	Decoction in water	Drink/Oral	04	1.40
				Amoebic dysentery	Roots			05	
				Diarrhea	Whole plant			12	
	<i>Euphorbia hirta</i> L.	Asthma-plant	Hendamniel debbi (Fufuldé)	Mycosis		Decoction in water	Drink/Oral	04	3.12
				Gonococci				02	
	<i>Ricinodendron heudelotii</i> (Baill.) Pierre ex Pax	African nut tree	Ndjansang	Varicella	Leaves	Crush	Rub/Topical	05	0.78
<i>Euphorbia prostrata</i> W. Ait.	Prostrate spurge			Gastric ulcers	Stem bark	Infusion/Decoction in water	Drink/Oral	03	1.40
				Malaria				04	
				Diarrhea	Roots			02	

	<i>Manniophytum fulvum</i> Müll. Arg.			Diarrhea	Stem bark	Decoction in water	Drink/Oral	03	0.46		
		<i>Gasso Nut</i>									
	<i>Ricinus cumunis</i> L.		Castor oil plant	Diarrhea	Root bark	Decoction in water	Drink/Oral	03	0.46		
Fabaceae	<i>Abrus precatorius</i> L.		Bead vine	Diarrhea	Stem bark	Decoction in water	Drink/Oral	02	0.31		
	<i>Albizia ferruginea</i> Benth.		-	Malaria	Stem bark	Decoction in water	Drink/Oral	03	0.46		
	<i>Pterocarpus soyauxii</i> Hooker		African Padauk	Intestinal-worms	Stem bark	Decoction in water	Drink/Oral	05	0.78		
	<i>Pterocarpus erinaceus</i> Poir.		Barwood	Boki (Fufuldé)	Amoebic dysentery	Leaves	Decoction in water	Drink/Oral	02	0.31	
	<i>Detarium microcarpum</i> Guill. & Perr.		Sweet Dattock	Koubehi (Fufuldé)	Skin infections	Seeds	Poultice	Rub/Topical	03	0.63	
Lamiaceae	<i>Mentha piperata</i> L.		Peppermint	Diarrhea	Leaves	Decoction in water	Drink/Oral	05	0.78		
	<i>Thymus vulgaris</i> L.		Thyme	Diarrhea	Whole plant	Decoction in water	Drink/Oral	04	1.72		
				Toothache			Teeth bath/Topical	02			
				Mycosis			Bath/Topical	05			
Lauraceae	<i>Persea americana</i> Mill.		Avocado tree	Tse-Pia	Diarrhea	Stem bark	Decoction in water	Drink/Oral	08	1.56	
					Intestinal-worms				04		
Liliaceae	<i>Allium cepa</i> Linn.		Onion	Anoussi	Cough	Fruit		Eat/Oral	10	2.97	
					Typhoid fever		-		09		
		<i>Aloe vera</i> Linn.		Aloe	Aloe vera	Malaria	Leaves	Maceration in water	Drink/Oral	05	1.72
Malvaceae	<i>Adansonia digitata</i> A L.		Baobab	Baobab	Diarrhea	Stem bark	Decoction in water	Drink/Oral	05	1.40	
					Amoebic dysentery				04		
		<i>Dissotis rotundifolia</i> (Sm.) Triana		Rock rose		Cough	Leaves	Decoction in water	Drink/Oral	03	0.46
Melastomataceae	<i>Azadirachta indica</i> Juss.		Neem tree		Rheumatism	Leaves	Decoction in water	Drink/Oral	03	1.25	
					Syphilis	Stem bark			05		
		<i>Carapa procera</i> DC.		Tallicoona oil tree	Engang (Ewondo)	Skin infections	Seed-oil	-	Ointment /Topical	03	1.09
		<i>Lovoa trichilioides</i> Harms		African Walnut		Intestinal-worms	Roots	Decoction in water	Rectal route	04	
						Toothache	Stem bark	Decoction in water	Teeth bath/Topical	02	0.31
Moraceae	<i>Ficus thonningii</i> Blume		Common wild fig	umBombe ( Zulu)	Intestinal-worms	Leaves	Maceration in water	Drink/Oral	03	0.46	
	<i>Musanga cecropioides</i> C. Sm. ex R. Br.		Umbrella tree	Asseng (Beti)	Vaginal infections	Sap	Maceration in water	Bath/Topical	02	0.78	
					Malaria	Stem bark	Decoction in water	Drink/Oral	03		
	<i>Milicia excels</i> (Welw.) C.C. Berg		African teak	Iroko	Intestinal-worms	Latex	Maceration in water	Drink/Oral	02	0.31	
Moringaceae	<i>Moringa oleifera</i> Lam		Moringa	Moringa	Toothache	Roots	-	Chew/Topical	14	3.59	
					Diarrhea	Stem bark	Maceration in water	Drink/Oral	04		
					Dermatitis	Seeds	Crush	Ointment/Topical	05		
Musaceae	<i>Musa paradisiacal</i> L.		Plantain banana tree	Tse-Keloung	Amoebic dysentery	Leaves of the bud	Infusion/Decoction in water	Drink/Oral	05	0.78	
Myrtaceae	<i>Eucalyptus grandis</i> Hill ex Maiden		Eucalyptus	Tse-doc	Malaria	Stem bark	Decoction in water	Drink/Oral	03	0.46	

	<i>Melaleuca alternifolia</i> (Maiden & Betche) Cheel	Tea tree		Scabies	Leaves	Crush	Rub/Topical	04	1.09
				Vaginal infections		Decoction in water	Bath/Topical	03	
	<i>Psidium guajava</i> (L.)	Guava tree	Tomtse	Toothache	Leaves	-	Chew/Topical	12	2.66
	<i>Syzygium aromaticum</i> (L.) Merr. & L.M.Perry	Clove	Ding Xiang (Chinese)	Malaria	Stem bark	Decoction in water	Drink/Oral	05	
				Viral infections	Flower buds	Decoction in water	Drink/Oral	02	1.40
Piperaceae	<i>Piper umbellatum</i> Linn.	Cow-foot leaf	Yawe (Nigeria)	Rheumatism	Leaves	Decoction in water	Drink/Oral	03	1.09
				Diarrhea				04	
Poaceae	<i>Cymbopogon citrates</i> (DC.) Stapf	Citronella	Fipagrassi	Dermatitis	Leaves	Crush	Rub/Topical	03	0.46
Rhamnaceae	<i>Zizyphus mauritiana</i> Lam.	Jujub tree	Didjemm	Wounds disinfection	Fruit	Poultice	Rub/Topical	03	0.46
Rubiaceae	<i>Morinda elliptica</i> (Hook. f.) Ridl.	India mulberry	Mengundu	Diarrhea	Wood	Decoction in water	Drink/Oral	05	1.25
				Dermatitis	Fruit	Decoction in water	Bath/Topical	03	
	<i>Morinda lucida</i> Benth.	Brimstone tree	kwakengue	Hepatitis	Roots	Decoction in water	Drink/Oral	03	1.25
				Malaria	Leaves			05	
Rutaceae	<i>Citrus sinensis</i> Linn. Osbeck	Orange tree	Tse-Poumà	Malaria	Leaves	Decoction in water	Drink/Oral	05	0.78
	<i>Crossopteryx febrifuga</i> (Afzel. ex G.Don) Benth	Common crown-berry	Fula-pulaar (Senegal)	Diarrhea	Roots	Decoction in palm wine	Drink/Oral	02	
				Rheumatism		Infusion in water		03	0.78
	<i>Citrus limon</i> Linn. Burm.	Lemon tree	Citron	Diarrhea		Decoction in water		03	2.34
				Chlamydia	Fruit	Infusion in water	Drink/Oral	08	
				Malaria				04	
	<i>Zanthoxylum xanthoxyloides</i> (Lam.) Zepern. & Timler	candlewood	Fasahuari (Haoussa)	Diarrhea	Fruit	Infusion in water	Drink/Oral	03	0.93
				Amoebic dysentery				03	
	<i>Clausena anisata</i> (Willd.) Hook.f. ex Benth.	Horsewood	Jumba (Bakweri)	Typhoid fever	Stem bark	Decoction in water	Drink/Oral	06	0.93
Solanaceae	<i>Slonatum torvum</i> Sw.	wild egg plant	Top Na Aka (Batoufam)	Mycosis	Stem bark			02	1.09
				Diarrhea	Leaves	Infusion in water	Drink/Oral	05	
Sterculiaceae	<i>Cola nitida</i> Schott & Endl	Cola tree	Tse-Stsre	Amoebic dysentery	Kola nuts	-	Eat/Oral	03	0.93
				Diarrhea				03	
Verbenaceae	<i>Stachytarpheta jamaicensis</i> (L.) Vahl	Brazilian tea	Barkiyar kusu (Haoussa)	Abscesses	Whole plant	Poultice	Rub/Topical	02	0.31
Vitaceae	<i>Cissus petiolata</i> Hook.	-	kihindhindi (Zigua)	Mouth mycosis	Roots	Infusion in water	Bath/Topical	04	0.62
Zingiberaceae	<i>Aframomum melegueta</i> K. Schum.	Grains of paradise	gûza sahrâwiya (Maroc)	Dermatitis	Seeds	Crush	Rub/Topical	06	0.93
	<i>Zingiber officinale</i> Rosc.	Ginger	Djidja	Dermatitis		Crush	Rub/Topical	03	1.56
				Cough	Roots	-	Eat/Oral	07	

**Table 2:** Infectious diseases inventoried

Infections	Frequency	Percentage
Malaria	121	18.93
Diarrhea	106	16.58
Intestinal- worms	65	10.17
Amoebic dysentery	46	7.19
Toothache	44	6.88
Scabies	31	4.85
Dermatitis	28	4.38
Rheumatism	27	4.22
Cough	25	3.91
Mycosis	24	3.75
Wounds disinfection	23	3.59
Typhoid fever	19	2.97
Vaginal infections	13	2.03
Syphilis	11	1.72
Skin infections	11	1.72
Chlamydia	8	1.25
Hepatitis	7	1.09
Gastric ulcers	7	1.09
Viral infections	6	0.93
Varicella	5	0.78
Pediculosis	3	0.46
Tuberculosis	3	0.46
Measles	2	0.31
Abscesses	2	0.31
Gonococci	2	0.31

**Table 3:** Plant parts used in traditional recipes

Plant parts used	Frequency	Percentage
Stem bark	204	31.92
Leaves	197	30.82
Roots	91	14.24
Fruits	59	9.23
Whole plant	35	5.47
Seeds	25	3.91
Latex	6	0.93
Nuts	6	0.93
Wood	5	0.78
Flowers	5	0.78
Resin	2	0.31
Aerial part	2	0.31
Sap	2	0.31

**Table 4:** Preparation methods

Preparation methods	Frequency	Percentage
Decoction in water	328	53.24
Infusion in water	118	19.15
Crush	65	10.55
Maceration in water	65	10.55
Poultice	35	5.68
Maceration in palm wine	3	0.48
Decoction in palm wine	2	0.32

**Table 5:** Modes of administration

Administration	Frequency	Percentage
Drink	414	64.78
Rub	102	15.96
Bath	45	7.04
Eat	35	5.47
Chew	29	4.53
Ointment	8	1.25
Rectal route	6	0.93

**Table 6:** Routes of administration

Administration route	Frequency	Percentage
Oral	453	70.89
Topical	180	28.16
Rectal route	6	0.93

**Extraction and time-kill analysis**

*Plant extracts and phytochemical screening*

The extraction yields and chemical constituents of hydro-ethanol extracts of plants tested are closed in **Table 7**. These results show that, the extraction yields are ranged between 4.60 (ex-tract of bulb of *Allium cepa*) and 14.23% (extract of stem barks of *Voacanga africana*). The chemical screening shown the relatively presence of metabolites compounds like: phenols, flavonoids, tannins, coumarins, anthraquinone, terpenoids, cardiac glycosides, anthocyanides and alkaloids, in the plants extracts.

**Inhibition parameters**

The results obtained from inhibition parameters closed in **Table 8** below show that, the MIC determined are ranged between 1 and 8 mg/mL and the MBC between 2 and 32 mg/mL. The values obtained from MBC/MIC ratio evaluation are ranged between 1 and 4.

**Time-kill assay**

The time kill antibacterial assay of plant extracts has given variable kinetics against *Escherichia coli* strain as seen in **Figure 1**. A significant decrease ( $P < 0.05$ ) in population of test organisms was observed with the increase in incubation time. The log reduction in viable cell count are ranged between 0.60 and 3.69  $\log_{10}$ cfu/mL after 6 hours of incubation, and between 0.13  $\log_{10}$ cfu/mL and 100% of inhibition after 12, 18, 24, 30, 36, 42 and 48 hours of incubation (**Tables 10 and 11**). At 1 x MIC, a total cell destruction has been obtained from *Chromolaena odorata*, *Euphorbia hirta* and *Enantia chlorantha* extracts after 18 hours of incubation, from *Alstonia boonei*, *Psidium guajava* and *Mammea africana* extracts after 24 hours, from *Voacanga africana*, *Moringa oleifera* and *Allium cepa* extracts after 30 hours, and from *Picalima nitida* extract after 42 hours of incubation. At 2 x MIC, a total cells killing has been obtained from *Euphorbia hirta*, *Enantia chlorantha*, *Allium cepa* extracts after 12 hours of incubation, from *Voacanga africana*, *Moringa oleifera*, *Psidium guajava*, *Chromolaena odorata* and *Alstonia boonei* extracts after 18 hours, from *Mammea africana* extract after 24 hours, and from *Picalima nitida* after 30 hours of incubation. On the other hand, relative to the initial cell number, the first times of incubation for which a bactericidal effect has been observed from the plant extracts ( $\log$  reduction  $\geq 3 \log_{10}$ cfu/mL) are defined as following:

**a.** After 6 hours of incubation, at 1 x MIC, bactericidal activity was observed from *Voacanga africana* and *Enantia chlorantha* extracts which are reduced viable initial cells to 6.03 at 3.37 and 3.19  $\log_{10}$ cfu/mL, respectively. On the other hand, bactericidal activity was obtained from *Moringa oleifera*, *Chromolaena odorata* and *Enantia chlorantha* extracts, at 2 x MIC, with cell reduction of 4.40, 3.80 and 4.80  $\log_{10}$ cfu/mL, respectively.

**b.** After 12 hours of incubation, log reduction in viable cell count varies between 0.13  $\log_{10}$ cfu/mL and 100% of inhibition. At 1 x MIC, bactericidal activity was obtained from *Chromolaena odorata*, *Mammea africana* and *Allium cepa* extracts with log decreasing in viable cells of 4.89, 4.15 and 3.16  $\log_{10}$ cfu/mL, respectively. This bactericidal action was observed at 2 x MIC with *Voacanga africana*, *Alstonia boonei*, *Psidium guajava* and *Mammea africana* which have exhibited log decrease of 3.65, 5.10, 5.10 and 4.71  $\log_{10}$ cfu/mL, respectively.

c. After 18 hours of incubation, at 1 x MIC, bactericidal activity was obtained with *Moringa oleifera* extract with log reduction of 4.69  $\log_{10}$ cfu/mL. At 0.5 x MIC bactericidal activity was observed from *Chromolaena odorata* and *Psidium guajava* extracts with reduction of 3.03 and 4.96  $\log_{10}$ cfu/mL, respectively.

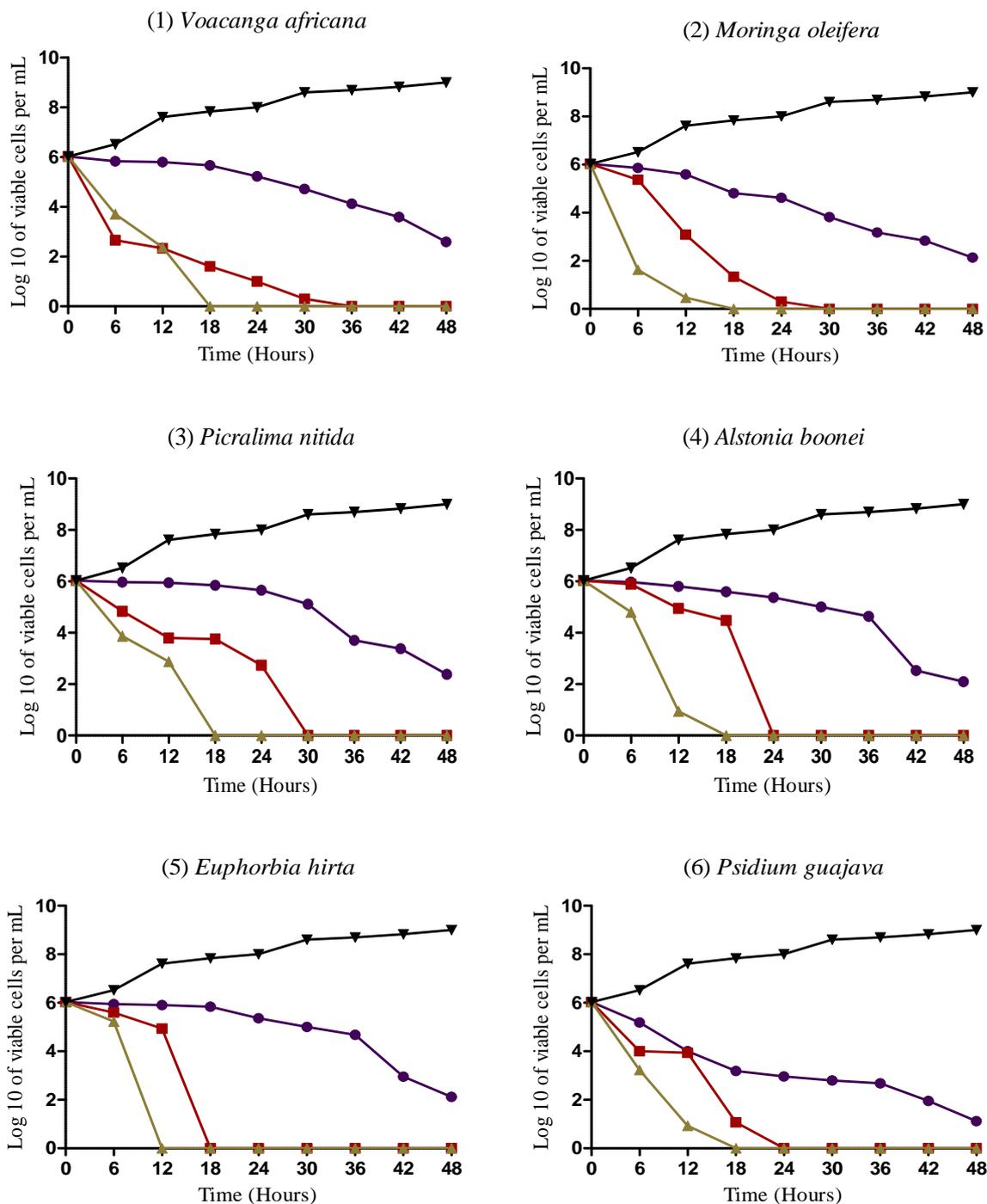
d. After 24 hours of incubation, *Picralima nitida* and *Psidium guajava* extracts were bactericidal with log reduction of 4.15 and 3.07  $\log_{10}$ cfu/mL, respectively, at 2 x MIC.

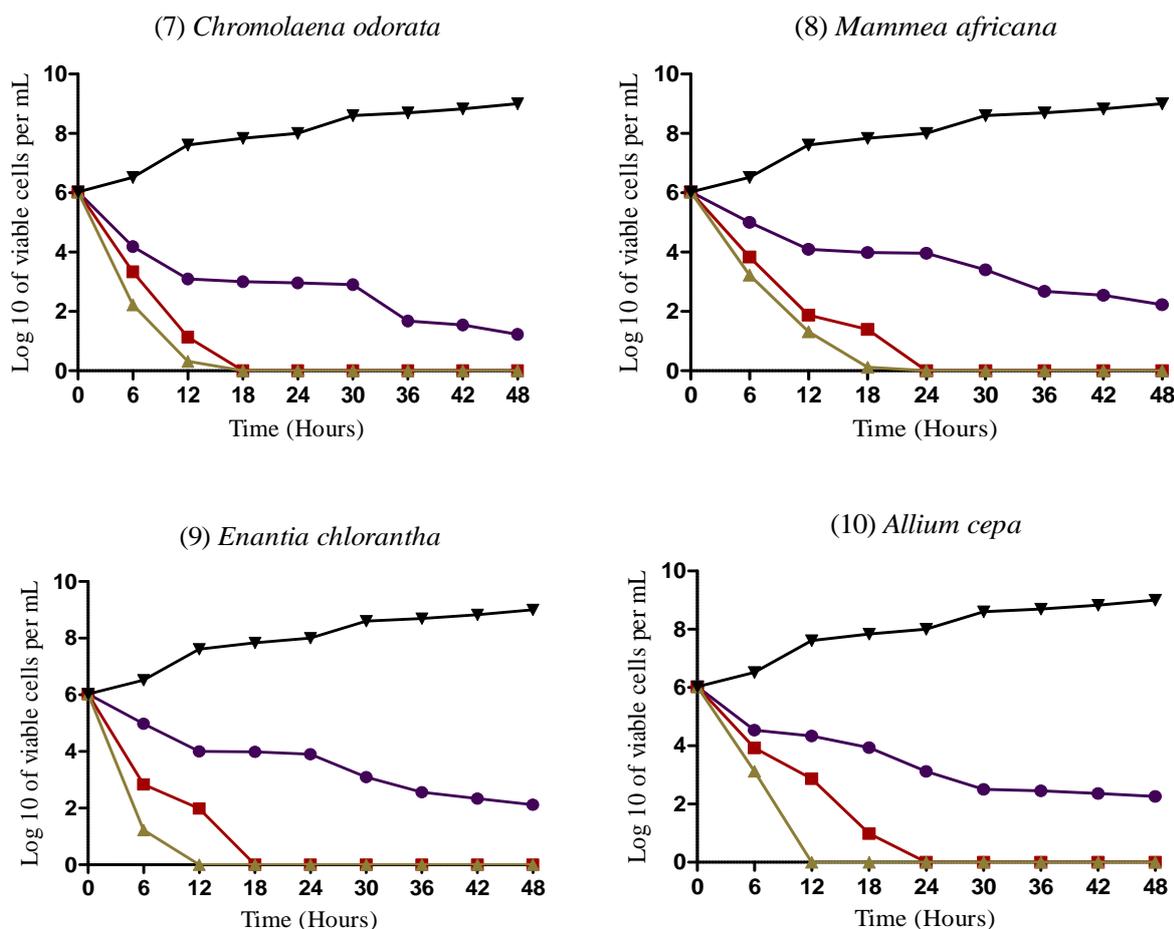
e. After 30 hours of incubation, *Allium cepa* extract was bactericidal at 0.5 x MIC with log decreasing of 3.53  $\log_{10}$ cfu/mL. The bactericidal activity was also obtained from *Picralima nitida* extract, at 1 x MIC, with log decreasing of 3.26  $\log_{10}$ cfu/mL.

f. After 36 hours of incubation, at 0.5 x MIC, bactericidal activity was obtained from *Mammea Africana* and *Enantia chlorantha* extracts with log reduction in viable cell count of 3.35 and 3.47  $\log_{10}$ cfu/mL, respectively.

g. After 42 hours of interaction, *Moringa oleifera*, *Picralima nitida*, *Alstonia boonei* and *Euphorbia hirta* with decreasing in log of 3.19, 3.50, 3.50 and 3.08  $\log_{10}$ cfu/mL, respectively, at 0.5 x MIC.

h. After 48 hours of incubation, at 0.5 x MIC, *Voacanga africana* extract was bactericidal with log reduction in cell count of 3.43  $\log_{10}$ cfu/mL.





**Figure 1:** Growth curves of *Escherichia coli* strain in Muller Hinton broth with 0 (control), 0.5, 1 and 2 times MIC of plant extract during 48 h of incubation.

## DISCUSSION

The ethno-pharmacological survey realized in this study has permitted to identify a total of 79 medicinal plants species listed in 41 families. These plants have been cited to be use in the treatment of about 25 infectious diseases among which, malaria, diarrhea and intestinal-worms were the most prevalent. This is related to the report of National Institute of the Statistics of Cameroon (INS) which was pointed out malaria and diarrhea for their high child mortality rate in Cameroon. Moreover, Global Burden of Disease Study (GBD)<sup>58</sup> has also cited malaria and diarrheal diseases to be among the first causes of premature death in Cameroon. According to the Fidelity level, *Voacanga africana*, *Chromolaena odorata*, *Moringa oleifera*, *Euphorbia hirta* and *Mammea africana* were the most cited plant species. Many methods have been mentioned to be use in the preparation of traditional curative recipes but, it is difficult to say precisely which method is effective because they were different from one traditional healer to another<sup>59</sup>. However, decoction and infusion have been mentioned to be the main modes of preparation. The main mode of administration of recipes was the oral route. This is in agreement with many previous ethnopharmacological studies which were also mentioned oral route to be the main mode of administration of traditional potions.

In Cameroon, diarrhea constitutes one of the mains causes of death in child population with 6 at 23 months old and there is not a strategic plan to fight against this disease<sup>60</sup>. Basing on the statistical data collected in Cameroonian health centers, diarrheal infections caused by *Escherichia coli* have become most prevalent and constitute an issue of Public Health concern. In this study, the sensitivity of *Escherichia coli* to hydro-ethanol extracts of the ten most cited plants has been evaluated *in vitro*. These plants were *Voacanga africana*, *Chromolaena odorata*, *Moringa oleifera*, *Euphorbia hirta*, *Mammea africana*, *Alstonia boonei*, *Picralima nitida*, *Psidium guajava*, *Enantia*

*chlorantha* and *Allium cepa*. The inhibition parameters determined are ranged between 1.00 and 8.00 mg/mL for the MIC, and between 2.00 and 32.0 mg/mL for the MBC. The best activity was obtained to hydro-ethanol leaves extract of *Chromolaena odorata* with MIC and MBC of 1.00 mg/mL and 2.00 mg/mL, respectively. Further, eighty percent (80%) of plant extracts tested have been bactericidal on bacterial strain with MBC/MIC ratio equal to 1 or 2. This bactericidal effect reflects the truth why local endogenous populations in west-Cameroon use these medicinal plants for their health need. Note that, some extracts of these plants have been reported in previous studies for their antibacterial properties (Table 12). Several authors have also investigated concerning the pharmacotoxicity of some of these plant species and the data obtained are available in literature.

To characterize the pharmacodynamic interaction between plant extracts and *Escherichia coli* strain, the time kill assay was performed over a period of 48 hours, with the reading time intervals of 6 hours. The bacterial cells were exposed to the increasing concentrations of plant extracts of 0.5 × MIC, 1 × MIC and 2 × MIC. The time kill kinetics obtained (Figure 1) have shown a significant decrease ( $p < 0.05$ ) in population of test organisms. The strength of bactericidal efficiency was time and dose dependent. For instance, after 18 hours of incubation, the cells were completely destroyed by *Euphorbia hirta* and *Enantia chlorantha* extracts, at 1 × MIC. The total reduction in viable cell count was obtained from these plant extracts after 12 hours, at 2 × MIC. Further, for 2 × MIC, the complete bactericidal action was observed for all the plant extracts after 24 hours of exposure while this one was observed after 30 hours for 1 × MIC and for over 48 hours at 0.5 × MIC. However, the trend of bactericidal activities show that after 18 hours of exposure, there was a high significant ( $p < 0.05$ ) percentage reduction of viable cell count for all the plant extracts, with log reduction ranged between 73.30% (- 4.42 log<sub>10</sub>cfu/mL) and 100% (- 6.03 log<sub>10</sub>cfu/mL), at 1 × MIC and at 2 × MIC.

**Table 7:** Extraction yield and chemical constituents of hydro-ethanol extracts of plants tested

Plant parts	Plant species	Extraction Yield (%)	Metabolite groups								
			Phenols	Flavonoids	Tannins	Coumarins	Anthraquinones	Terpenoids	Cardiac glycosides	Anthocyanins	Alkaloids
Stem bark	<i>Voacanga africana</i>	14.23	++	++	+	-	++	+	+	++	++
	<i>Moringa oleifera</i>	11.45	++	+	++	+	+	++	+	+	+
	<i>Picalima nitida</i>	13.67	++	++	+	+	++	+	++	++	+
	<i>Alstonia boonei</i>	13.42	+++	++	++	-	-	+	++	+	++
Leaves	<i>Euphorbia hirta</i>	08.22	++	+	+	+	+	++	+	+	++
	<i>Psidium guajava</i>	09.44	+++	++	+	+	++	+	++	+	+
	<i>Chromolaena odorata</i>	10.23	++	++	+	++	+	++	+	+	+
	<i>Mammea africana</i>	11.97	+	+	++	+	+	+	+	++	-
	<i>Enantia chlorantha</i>	09.54	+++	+	++	++	++	+	+	++	+
Bulb	<i>Allium cepa</i>	04.60	++	++	+	-	+	++	+	+	+

-: not detected; +: present in small amount; ++: present in average concentration; +++: present in high amount.

**Table 8:** Inhibition parameters of hydro-ethanol extracts of plant parts selected against *Escherichia coli*

Plant parts	Plant species	Inhibition parameters			Action
		MIC	MBC	MBC/MIC	
Stem bark	<i>Voacanga africana</i>	4.00	8.00	2	Bactericidal
	<i>Moringa oleifera</i>	2.00	2.00	1	Bactericidal
	<i>Picalima nitida</i>	4.00	4.00	1	Bactericidal
	<i>Alstonia boonei</i>	4.00	4.00	1	Bactericidal
Leaves	<i>Euphorbia hirta</i>	4.00	8.00	2	Bactericidal
	<i>Psidium guajava</i>	8.00	32.0	4	Bacteriostatic
	<i>Chromolaena odorata</i>	1.00	2.00	2	Bactericidal
	<i>Mammea africana</i>	4.00	16.0	4	Bacteriostatic
	<i>Enantia chlorantha</i>	2.00	2.00	1	Bactericidal
Bulb	<i>Allium cepa</i>	4.00	8.00	2	Bactericidal
Reference	Gentamycin	0.06	0.06	1	Bactericidal

MIC: Minimal inhibitory concentration (mg/mL); MBC: Minimal bactericidal concentration (mg/mL)

**Table 10:** Average log reduction in viable cell count between 6 and 24 h of incubation, in presence of hydro-ethanol plant extracts at 0.5 x MIC, 1 x MIC and 2 x MIC

Hydro-ethanol extracts of plant species	Reduction in cell counts ( $\Delta \log_{10} \text{cfu/mL}$ )											
	$\Delta \log_{10} \text{cfu/mL}$ at 6 h			$\Delta \log_{10} \text{cfu/mL}$ at 12 h			$\Delta \log_{10} \text{cfu/mL}$ at 18 h			$\Delta \log_{10} \text{cfu/mL}$ at 24 h		
	0.5 x MIC	1 x MIC	2 x MIC	0.5 x MIC	1 x MIC	2 x MIC	0.5 x MIC	1 x MIC	2 x MIC	0.5 x MIC	1 x MIC	2 x MIC
<i>Voacanga africana</i>	- 0.19	- 3.37*	- 2.32	- 0.23	- 3.69*	- 3.65*	- 0.36	- 4.42*	TI	- 0.80	- 5.03*	TI
<i>Moringa oleifera</i>	- 0.17	- 0.66	- 4.40*	- 0.43	- 2.94	- 5.56*	- 1.22	- 4.69*	TI	- 1.41	- 5.73*	TI
<i>Picalima nitida</i>	- 0.06	- 0.14	- 1.23	- 0.23	- 1.08	- 2.08	- 0.43	- 1.55	- 2.27	- 0.66	- 2.57	- 4.15*
<i>Alstonia boonei</i>	- 0.06	- 0.14	- 1.23	- 0.23	- 1.08	- 5.10*	- 0.43	- 1.55	TI	- 0.66	TI	TI
<i>Euphorbia hirta</i>	- 0.08	- 0.43	- 0.80	- 0.13	- 1.09	TI	- 0.19	TI	TI	- 0.67	TI	TI
<i>Psidium guajava</i>	- 0.84	- 2.03	- 2.80	- 2.03	- 2.09	- 5.10*	- 2.84	- 4.96*	TI	- 3.07*	TI	TI
<i>Chromolaena odorata</i>	- 1.84	- 2.69	- 3.80*	- 2.93	- 4.89*	- 5.71*	- 3.03*	TI	TI	- 3.07*	TI	TI
<i>Mammea africana</i>	- 1.03	- 2.19	- 2.80	- 1.93	- 4.15*	- 4.71*	- 2.04	- 4.63*	- 5.91*	- 2.07	TI	TI
<i>Enantia chlorantha</i>	- 1.05	- 3.19*	- 4.80*	- 2.03	- 4.04*	TI	- 2.04	TI	TI	- 2.13	TI	TI
<i>Allium cepa</i>	- 1.49	- 2.1	- 2.90	- 1.69	- 3.16*	TI	- 2.09	- 5.04*	TI	- 2.91	TI	TI
Growth control	0.49			1.58			1.81			1.97		

- represents the decreasing in viable cells counts compared to initial inoculums, \* represents bactericidal effect and TI represents the total inhibition

**Table 11:** Average log reduction in viable cell count between 30 and 48 h of incubation, in presence of hydro-ethanol plant extracts at 0.5 x MIC, 1 x MIC and 2 x MIC

Hydro-ethanol extracts of plant species	Reduction in cell counts ( $\Delta \log_{10} \text{cfu/mL}$ )											
	$\Delta \log_{10} \text{cfu/mL}$ at 30 h			$\Delta \log_{10} \text{cfu/mL}$ at 36 h			$\Delta \log_{10} \text{cfu/mL}$ at 42 h			$\Delta \log_{10} \text{cfu/mL}$ at 48 h		
	0.5 x MIC	1 x MIC	2 x MIC	0.5 x MIC	1 x MIC	2 x MIC	0.5 x MIC	1 x MIC	2 x MIC	0.5 x MIC	1 x MIC	2 x MIC
<i>Voacanga africana</i>	- 1.31	TI	TI	- 1.90	TI	TI	- 2.43	TI	TI	- 3.43*	TI	TI
<i>Moringa oleifera</i>	- 2.21	TI	TI	- 2.85	TI	TI	- 3.19*	TI	TI	- 3.89*	TI	TI
<i>Picalima nitida</i>	- 1.03	- 3.26*	TI	- 1.39	- 4.10*	TI	- 3.50*	TI	TI	- 4.03*	TI	TI
<i>Alstonia boonei</i>	- 1.03	TI	TI	- 1.39	TI	TI	- 3.50*	TI	TI	- 3.93*	TI	TI
<i>Euphorbia hirta</i>	- 1.03	TI	TI	- 1.35	TI	TI	- 3.08*	TI	TI	- 3.91*	TI	TI
<i>Psidium guajava</i>	- 3.23*	TI	TI	- 3.35*	TI	TI	- 4.08*	TI	TI	- 4.91*	TI	TI
<i>Chromolaena odorata</i>	- 3.13*	TI	TI	- 4.35*	TI	TI	- 4.48*	TI	TI	- 4.80*	TI	TI
<i>Mammea africana</i>	- 2.63	TI	TI	- 3.35*	TI	TI	- 3.48*	TI	TI	- 3.77*	TI	TI
<i>Enantia chlorantha</i>	- 2.93	TI	TI	- 3.47*	TI	TI	- 3.69*	TI	TI	- 3.91*	TI	TI
<i>Allium cepa</i>	- 3.53*	TI	TI	- 3.58*	TI	TI	- 3.67*	TI	TI	- 3.77*	TI	TI
Growth control	2.57			2.67			2.80			2.97		

- represents the decreasing in viable cells counts compared to initial inoculums, \* represents bactericidal effect and TI represents the total inhibition

**Table 12:** Some previous antibacterial reports for plant species tested in this study

Plant species	Phytochemistry	Some activity values
<i>Voacanga africana</i>	Alkaloids, anthraquinone, cardiac glycosides, phenols, phlobatanins, starch and tannins <sup>12</sup> . Alkaloids identified: Voacamine, voacangine, voacangarine, voacordine and vobtusine.	Methanol extract of seed: Diameter of inhibition zone of 7 mm against <i>Staphylococcus aureus</i> and 11 mm against <i>Enterococcus hirae</i> , at 1.5 mg dried extract/disc <sup>13</sup> .
<i>Moringa oleifera</i>	Saponins, Tannins, Flavonoids, Alkaloids <sup>14, 15</sup> . niazimicin, benzyl isothiocyanate, and 4-( $\alpha$ -L-rhamnopyranosyloxy) benzyl glucosinolate, Anthonine, Spirochin, Alkaloid Moringine, b-sitosterol, Quecetin and kaempferol, Niazimicin, 4-( $\alpha$ -L-rhamnosyloxybenzyl)-omethyl thiocarbamate, niazinin A, niazinin B, niazimicin <sup>16, 17, 18, 19, 20, 21</sup>	Aqueous Fresh leaves extracts : Diameter of inhibition zone of $27.5 \pm 0.21$ mm against <i>Pseudomonas spp.</i> , $15.00 \pm 0.34$ mm against <i>Pseudomonas aeruginosa</i> and $17.25 \pm 0.14$ mm against <i>Bacillus subtilis</i> . Fresh leaf juice: MIC value of 1.25 $\mu$ L.disc-1 against <i>Shigella shinga</i> and <i>Bacillus subtilis</i> <sup>22</sup> . Methanol fruit extract: Inhibition diameter of 22 mm on <i>Pseudomonas aeruginosa</i> , 19 mm on <i>Bacillus cereus</i> , 16 mm on <i>Staphylococcus aureus</i> and <i>Bacillus subtilis</i> , at 100 $\mu$ g/disc <sup>23</sup> . Aqueous stem bark extract: MIC value of 3.0 mg/mL against <i>Staphylococcus aureus</i> , <i>Escherichia Coli</i> , <i>Salmonella typhimurium</i> and <i>Pseudomonas aeruginosa</i> . Aqueous seeds' coat extract: MIC of 0.5 mg/mL against <i>Escherichia coli</i> , <i>Staphylococcus epidermidis</i> and <i>Salmonella typhimurium</i> <sup>24</sup> . Seed extracts: MIC and MBC of 0.025 mg/mL against <i>Escherichia Coli</i> , MIC of 0.049 mg/mL against <i>Pseudomonas aeruginosa</i> <sup>25</sup> .
<i>Picalima nitida</i>	Polyphenols, Cardiac Glycoside, Reducing sugars, Glycosides, Tannins, Ascorbic acid, Flavonoids, Alkaloids, Carbohydrates, Steroids, Glycolipid, Saponins, Protein, Terpenoids <sup>26, 27, 28</sup> . Some molecules characterized: 2,6-bis (1,1-dimethylethyl)-4-methyl phenol, sulfurous acid butyl cyclohexylmethyl ester, alpha-methyl mannofuranoside, hexadecanoic acid, methyl ester, 7-octadecenoic acid, methyl ester, N,N-dimethyl dodecanamide and N,N-dimethyl decanamide <sup>29</sup> .	Methanol extract: MIC values of 800 and 200 $\mu$ g/mL against <i>Shigella dysenteriae</i> type I and <i>Bacillus cereus</i> , respectively, and of 3.12 $\mu$ g/mL against <i>Escherichia coli</i> and <i>Staphylococcus aureus</i> , respectively <sup>27</sup> . Ethanol roots extract: Diameter of inhibition zone of $20 \pm 2.0$ , $16 \pm 1.73$ and $14 \pm 1.73$ mm against <i>Staphylococcus aureus</i> ATCC 12600, <i>Pseudomonas aeruginosa</i> ATCC 10145 and <i>Bacillus subtilis</i> ATCC 6051, respectively <sup>28</sup> .
<i>Alstonia boonei</i>	Saponins, General Glycosides, Flavonoids, Terpenoids and steroids Alkaloids <sup>30</sup>	Ethanol root extract : MIC of 0.98 mg/ml on <i>Staphylococcus aureus</i> , 2.68 mg/ml on <i>Pseudomonas aeruginosa</i> and 2.29 mg/mL on <i>Escherichia coli</i> <sup>30</sup>
<i>Euphorbia hirta</i>	Saponins, Tannins, Flavonoids, Alkaloids, Carbohydrates, Sterols, Steroids, Acidic compounds, Glycosides, Anthraquinone, Phenols, Terpenoids <sup>31, 32, 33</sup>	Ethanol extract of fresh leaves : MIC of 3.40 mg/mL on <i>Escherichia coli</i> , 0.27 mg/mL on <i>Pseudomonas aeruginosa</i> , 0.64 mg/mL on <i>Staphylococcus aureus</i> and 1.87 mg/ml on <i>Shigella dysenteriae</i> <sup>34</sup> Methanol leaves extract : MIC of 3.12 mg/mL on <i>E. coli</i> <sup>2</sup>
<i>Psidium guajava</i>	Carbohydrates, Reducing sugar, Alkaloids, Cardiac glycoside, Saponins, Tannins, Proteins, Oil, Steroids, Terpenoids <sup>35</sup> . Molecules isolated: Morin-3-O-lyxoside, Morin-3-O-arabinoside, Quercetin-3-O-arabinoside, Quercetin <sup>36</sup> .	Methanol leaves extract: Diameter of inhibition zone of $19.60 \pm 10.70$ mm against <i>Staphylococcus aureus</i> and of $16.70 \pm 9.50$ mm against <i>Escherichia coli</i> , at 100 $\mu$ g/mL <sup>37</sup> . Acetone extract of leaves: MIC of 0.312 mg/mL on <i>Streptococcus suis</i> and <i>Pasteurella multocida</i> <sup>38</sup> . Water and methanol leaves extracts: MIC value of 0.156 mg/mL against <i>Pasteurella multocida</i> and of 5 mg/mL against <i>Escherichia coli</i> <sup>38</sup> . Methanol leaves extract: MIC of 850 $\mu$ g/mL on <i>Vibrio cholera</i> <sup>39</sup> . Methanol stems bark extract: MIC and MBC of 62.5 $\mu$ g/mL against methicillin-resistant <i>Staphylococcus aureus</i> <sup>35</sup> .
<i>Chromolaena odorata</i>	Essential oil (Mains constituents: a-pinene, pregeijerene, geijerene, b-pinene, germacrene-D) <sup>40</sup> , Saponins, Tannins, Flavonoids, Glycosides, Anthraquinone, Phenols, Triterpenoids <sup>41</sup> . Some molecules isolated: 3',4',5,6,7-Pentamethoxyflavone and 4',5,6,7-Tetramethoxyflavone <sup>42</sup> , 5-Hdroxy 6,7,3',4'-tetramethoxyflavone, Dihydroxytrimethoxychalcone, <i>p</i> -Hydroxybenzoic acid, Pentaethoxyflavanone, Rhamsetin, Vanillic acid <sup>43</sup> , Sinensetin, 2',4-dihydroxy-4',5',6'-trimethoxychalcone, Scutellarein tetramethyl ether <sup>44</sup> , Lycopsamine, 3'-Acetyl rinderine, Rinderine and Echinatine <sup>45</sup> .	Essential oil: MIC of $1.28 \pm 0.06$ mg/mL on <i>Staphylococcus aureus</i> ATCC 25923 <sup>40</sup> . Acetone leaves extract: Diameter of inhibition zone of 12.6 mm against <i>E.coli</i> and <i>S. aureus</i> , at 1 mg/mL; Water leaves extract: Diameter of inhibition zone of 32.3 mm on <i>E. coli</i> <sup>47</sup> ; MIC of 0.25 mg/ml, 0.125 mg/ml on <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> , respectively <sup>46</sup> . Aqueous extract and ethyl acetate extract: MIC of 1.00 mg/mL on <i>Staphylococcus aureus</i> ; Ethanol extract: zone of inhibition of 37.7 mm on <i>Salmonella typhi</i> ; Water extract: zone of inhibition of 32.3 mm on <i>Escherichia coli</i> . Dichloromethane and Butanol leaves extracts: MIC of 0.156 mg/mL and 0.312 mg/mL on <i>Vibrio cholerae</i> , respectively <sup>42</sup>
<i>Mammea africana</i>	Coumarins, Flavonoids, Steroids, Terpenes <sup>48</sup> , glycosides, saponins. Mammeisin, 5,7-Dihydroxy-6-(3-methylbut-2-enyl)-8-(2-methyl-1-oxobutyl)-4-n-propyl-2H-[1]benzopyran-2-one <sup>49</sup> .	Methanol stem extract : Diameter of inhibition zone of 13 mm against <i>Staphylococcus aureus</i> <sup>48</sup>
<i>Enantia chlorantha</i>	Reducing Sugar, Saponins, Alkaloid, Phenols, Flavonoids, Glycosides <sup>50, 51</sup> .	Methanol stem bark extract: Diameter of inhibition zone of 25 mm against <i>Staphylococcus aureus</i> , 18 mm against <i>Streptococcus Pyogen</i> and 16 mm against <i>Escherichia coli</i> . Ethanol Stem Bark extract: Diameter of inhibition zone of 28 mm against <i>Staphylococcus aureus</i> , 25 mm against <i>Streptococcus Pyogen</i> , 18 mm against <i>Shigella sonnei</i> and 25 mm against <i>Escherichia coli</i> <sup>52</sup> .
<i>Allium cepa</i>	Flavonoids, Phenols <sup>53</sup> . Alkaloids, Cardiac glycoside, Steroids, Terpenoids, resins, organosulfur compounds <sup>54</sup> Thiosulfonates <sup>55</sup>	Essential oil : Diameter of inhibition zone of $38.2 \pm 1.09$ mm on <i>Bacillus cereus</i> , $9.5 \pm 0.21$ mm on <i>Salmonella enteritidis</i> and $9.5 \pm 0.10$ mm on <i>Escherichia coli</i> <sup>56</sup> . Bulbs of red variety of <i>Allium cepa</i> : Diameter of inhibition zone of 25 mm against <i>Bacillus subtilis</i> and 12 mm against <i>Escherichia coli</i> . Bulbs of white variety of <i>Allium cepa</i> : Diameter of inhibition zone of 27 mm against <i>Bacillus subtilis</i> and 13 mm against <i>Escherichia coli</i> <sup>57</sup> .

The time kill kinetics of some of the plant species tested in this study has been also evaluated in previous reports. It is the case of n-hexane extract of *Moringa oleifera* seeds which shown the completely destruction of the *Escherichia coli* cells, at  $0.5 \times \text{MIC}$  (0.0125 mg/mL), after the first 30 minutes of incubation<sup>61</sup>. The antibacterial activity obtained from the plants experimented in this study has been attributed to theirs metabolites contain.

Medicinal plants constituted a tank of bioactive antibiotics which has been for the long time a major source of new antibiotics. Many investigators have evaluated the therapeutic properties of some plant species identified in this work and several compounds have been isolated against serious infectious organisms. The compounds such as 2,6-bis(1,1-dimethylethyl)-4-methyl phenol and 3',4',5,6,7-Pentamethoxyflavone isolated respectively from *Picralima nitida* and *Chromolaena odorata* extracts have been reported for their interesting antibacterial activities<sup>29, 42</sup>. The plants may prove to be a rich source of compounds with possible antimicrobial activities but further research is necessary to determine the identity of the antimicrobial compounds from within these plants and also to determine their full spectrum of efficacy<sup>62, 63</sup>. Further, some researchers have showed the relationship between the traditional uses of plants species and theirs *in vitro* antimicrobial properties.

The antimicrobial activity of plant extracts tested in this study has been attributed to their chemical composition. In fact, the qualitative phytochemical screening lead on these plant extracts has permitted to detect metabolite groups like Phenols, flavonoids, tannins, coumarins, terpenoids, anthraquinones, cardiac glycosides, anthocyanides and alkaloids. Several studies have been reported to investigate the killing mechanism involved during the exposition of microbial cells to these metabolite compounds. The cell damages may carry out on cell membrane what lead to the lost of cellular materials and organelles from the cell cytoplasm. Metabolites like flavonoids have been reported for their ability to complex with the polypeptides of microbial cell wall<sup>64</sup>. Many others mechanisms are available in the literature. Since the plant appears to have a broad spectrum of action, they could be useful in antiseptic/disinfectant formulations and in chemotherapy<sup>34</sup>. The phytochemical screening of the medicinal plants tested of this work reveals it as a source of antimicrobial agents.

Since the 1960s, the Cameroonian authorities have done a lot to improve the health level of the population<sup>65</sup>. Despite this effort, the Cameroonian care financing know a serious difficulty binding to the economical crisis<sup>66</sup>. Until today, traditional medicine remains predominant compared to modern medicine in Cameroon. The use of plant derivatives as an alternative to the modern drugs is a current practice. In Cameroonian rural and urban zones, many medicinal and aromatic plants are known for their effectiveness against various infections. This study realized in Bandjoun village (La'Djo) has permitted the inventory of some of them which can be used in alternative and complementary medicine or serve like sources of future antibiotics. Furthermore, many researches confirming the anti-infectious values of most of the plants identified in this study are available in the literature.

## CONCLUSION

Because of the difficulty in accessibility and in efficiency of usually antibiotics, medicinal plants have always been investigated like a major source of new antimicrobial entities. Most bioactive substances have been isolated from plants and their scientific validation has been performed concerning their safety and efficacy properties against microbes. In this study, 79 medicinal plant candidates have been identified in indigenous system of medicines in west-Cameroon region. These plants have been cited in treatment of various infectious ailments among which malaria, diarrhea and intestinal-worms have been the most cited. Extracts of some of these plants have been tested *in-vitro* against *Escherichia coli* strain and their anti-bacterial properties were confirmed; in agreement with previous reports. According to the information collected concerning their curative

properties, these plants could be the target of future scientific researches for discover of innovative antimicrobial drugs. This work is our contribution in the no limit researches which occur worldwide for discover of new therapeutic agents.

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