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Anti-mycobacterial efficacy of three essentials oils from medicinal plants currently used traditionally to treat tuberculosis in Cameroon

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Abstract
The designed study carries out an ethnopharmacology survey of medicinal plant used to treat tuberculosis and asses theanti-mycobacterial efficiency of three of their plant essential oils against three strains of *Mycobacterium tuberculosis*. The ethnopharmacological study was carried out in two localities of Nkam Division where plants specimens were collected. Three plants were selected, and their essential oils were obtained by hydrodistillation and analysed by Gas Chromatography and Gas Chromatography-Mass Spectrometry. Anti-mycobacterial activity of these essential oils was evaluated against four resistant isolates using the microdilution method. Twelve plants were collected; the most used were *Drypetesgoss weileri* S. Moore (*Putranjivaceae*), *Pentadiplandra brazeana* Baill (*Pentadiplandraceae*) and *Allium sativum*L (*Amaryllidaceae*). Benzyl isothiocyanate was the major component in *D. gossweileri* and *P. brazzeana*essential oils at 91.27% and 96.00% respectively and with 2-methylpropenyl trisulfide at 51.02% methylallyltrisulfide (12.8%), diallylsulfide (11.1%) for *A. sativum*. The essential oils of *A. sativum* and *P. brazzeana* essential oil exhibited it higher activity with minimum inhibitory concentrations (MIC) of 78.12 µg/mL and 312.50 µg/mL against extensively resistant isolate while highest activity of *D. gossweileri* was against Isoniazid resistant isolate with MIC of 156.25 µg/mL. The results justified the traditional usage of theseplant by Nkam populations for treating tuberculosis cases.

Keywords: Ethnopharmacological survey, essential oils, anti-mycobacterial efficacy

1. Introduction
Tuberculosis (TB) is an airborne infectious disease caused by the complex of *Mycobacterium tuberculosis* bacteria which generally affects lungs [1, 2]. The World Health Organization (WHO) estimated 9.3 million new cases and 13.7 million chronic active cases responsible for 1.7 million deaths worldwide yearly [3]. Despite the progress in TB diagnosis, treatment and prevention efforts, the proportion of TB cases living with HIV is still on rise in Africa region where about 81% of people had been notified among men, women and children [3]. The cause being that, the majority of *Mycobacterium* species are resistant to the most widely used therapeutic agents in TB treatment. Thus, there is an urgent need for new efficient antimycobacterial agents to replace or supplement those currently used. Plant components have received considerable attention as potential anti-TB agents during the last years with recent reviews showing their anti-mycobacterialpotency [4]. Most *in vitro* and *in vivo* studies presented the therapeutic effect of new plant components against resistant and non-resistant *Mycobacterium tuberculosis* [5, 6, 7]. In Cameroon, many people often used auto medication by plant traditional medicines to avoid the expensive cost of drugs or illness recidivism; such plants should be identified and screened in the basis of traditional knowledge to show their efficacy in the treatment of TB. Some reports mentioned the antimicrobial effects of Cameroonian plant but just few have been focusing on their antimycobacterial and fewer on the antimycobacterial activities of their essential oils [8, 9, 10]. This work reports the *in vitro* antimycobacterial evaluation of three essential oil plants identified during an ethnopharmacological survey carried out in two localities of Nkam Division in Littoral Region of Cameroon.

2. Materials and methods

2.1. Ethnopharmacological survey

2.1.1. Area of study

Between 4°0-5°10 Latitude North and 9°35-10°35 Longitude East, the Nkam Division is one of the four Departments that constitute the Littoral region in Cameroon and has five subdivisions as show in Figure 1. It is localized along three rivers: the Makombè, Nkam and Dibamba with the predominant soil isrich in iron lithosol covered by a huge forest vegetation. This site has high humidity climate with an average temperature between 23 and 29 °C and annual precipitation between 2900 and 3000 mm. It covers an area of 6291 km^2 with total population of 66,979 inhabitants, and as in many other localities of Cameroon, people use plant traditional medicine more than conventional medicine. The study has been carried out in two localities of Nkam Division Yabassi and Nkondjock subdivisions, with the accord of the traditional rulers (chiefs and quarter heads) permitting or support our study.

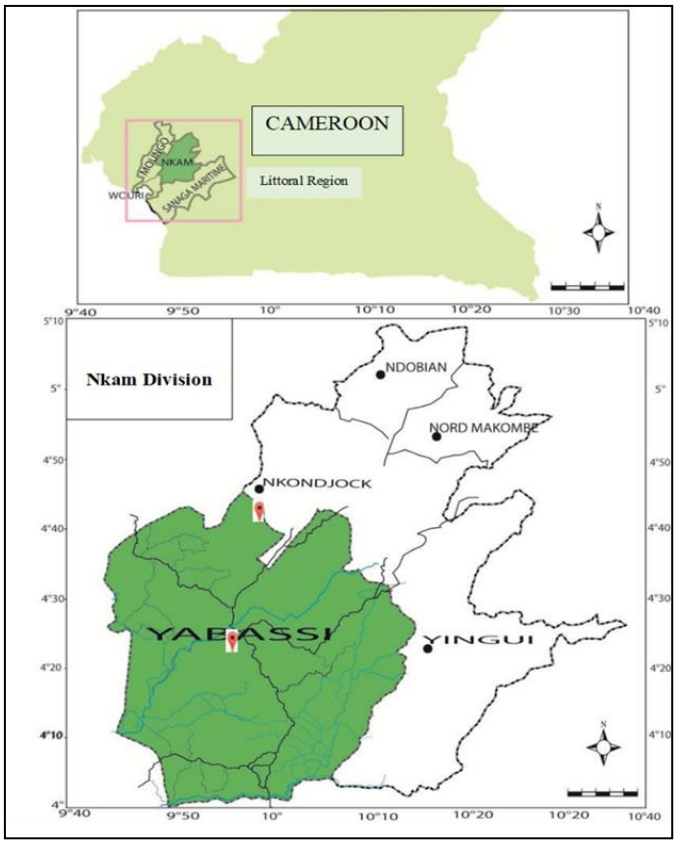

Fig 1: Geographical localization of Nkam and Nkondjock subdivisions in Littoral Region

2.1.2. Collection of ethnopharmacological information and plant sampling

The ethnobotanical information of plant specimens traditionally use for the treatment of tuberculosis were collected using the method described by Jovel *et al.*, consisting of general conversation and question aires [11]. The respondents were traditional healers, herb sellers and other villagers who had practical knowledge in the use (recipe and administration route) of medicinal plants for the treatment of tuberculosis uses. Data collected included some vernacular or local names of plants, parts of the plant used and the methods of preparation and administration. The specimens of medicinal plants indicated by informants were collected during the period of October and November 2015, identified at the National Herbarium. The frequency of the plants commonly used was calculated and the essential oils of the most recurrent plants were extracted and subjected to antimycobacterial tests.

2.2. Essential oil extraction Procedure

The essential oils of each plant materiel were extracted by hydrodistillation using a Clevenger-type apparatus for 5 h then, dried over anhydrous sodium sulfate and further stored at 4 °C until used. The extraction yields were calculated as the ratio of the mass of essential oil to the mass of the starting plant material expressed as a percentage.

2.3. Chemical Analysis of essential oils

2.3.1. Essential oils Analysis

Essential oils were analyzed by Gas Chromatography (GC) and Gas Chromatography coupled with Mass Spectrometry (GC-MS) as described by Agnaniet *et al.* [12].

2.3.2. Gas Chromatography (GC). GC

Analysis was performed on a Varian gas chromatograph, model CP-3380, with flame ionization detector containing two silica capillary columns: HP5 J&W Agilent (5%-Phenyl-methylpolysiloxane) capillary column (30 m × 0.25 mm i.d. × 0.25 µm film) and Supelcowax 10 (polyethylene glycol) fused capillary column (30 m × 0.25 mm i.d. × 0.25 µm film); N_2 was the carrier gas at 0.8 mL/min; injection type 0.1 µL of pure sample, split ratio 1:100; injector temperature 220 °C, detector temperature 250 °C; temperature program 50-200 °C at 5 °C/min, then kept at 200 °C for 10 min. The linear retention indices of the components were determined relative to the retention times of a series of n-alkanes. The entire set up was coordinated by Chromeleon (version 7.4) software system that ensured its functioning and follow-up of the chromatographic analysis.

2.3.3. Gas Chromatography-Mass Spectrometry (GC/MS). GC-MS

Analyses were performed using a Hewlett Packard 5890 II gas chromatograph, interfaced with a quadrupole detector (Model 5972) and equipped with a HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25µm). Helium was the carrier gas, at a flow rate of 0.6 mL/min. Injector and MS transfer line temperatures were 220 °C and 250 °C, respectively. The oven program temperature was the same as that used in the GC-FID analyses. Diluted samples (10:100 in CH_2Cl_2, v/v) of 1 µL were injected manually and in a split mode (1:100). The MS was operated in the EI mode at 70 eV, in the m/z range 35–300; electron multiplier 1460 eV; scan rate, 2.96 scan/s.

2.3.4. Qualitative Analysis

The identification of the constituents was assigned on the basis of a comparison of their relative retention indices, calculated with reference to a series of n-alkanes (C_9–C_{22}), and their mass spectra with those of the standards (for main components). Those found in the literature and supplemented by the NBS75K database and Wiley 7th NIST 2014 EPA/NIH Mass Spectral Library Upgrade (provided by Hewlett Packard with the GC/MS control and data processing software) [13, 14].

2.4.5. Quantitative Analysis

The percentage composition of the essential oils was computed by the normalization method from the GC-FID peak areas, assuming an identical mass response factor for all compounds [13, 14].

2.4. Anti-mycobacterial Activities

2.4.1. Chemical Reagents and Solvents

Glycerol and Tween 80 were purchased from Sigma-Aldrich (France). The Middle brook 7H9 OADC supplement and Alamar Blue were purchased from Becton Dickinson (USA). All other reagents and solvents were of analytical grade.

2.4.2. Essential oils and Anti-tuberculosis

Drugs Solutions. Stock solutions of essential oils were dissolved in the 7% tween 80 solution at the concentration of20000 µg/ mL. Stock solutions were kept at -20 °C until use. Standard anti-tuberculosis agents: Isoniazid (INH) and Rifampicin (RIF) were procured from commercial source (Becton Dickinson and company Spark, U.S.A). Stock solutions were prepared in sterile distilled water at 1000 µg/mL and sterilized by filtering through 0.20 µm membrane filter (Minisart).

2.4.3. Mycobacterium Strains and Growth Conditions

Four *Mycobacterium tuberculos* is resistant strains were used for this study. Two of them, AC_{45} an Ionized resistant strain and AC_{79} a Rifampicin resistant strain, were isolated from patients in a tuberculosis surveillance framework in the South region of Cameroon. Their sensitivity to antibiotics has been provide using Line Probe Assay genotype (Hain Life science kits) test at the Laboratory for Tuberculosis Research of the Biotechnology Centre of University of Yaoundé I. Meanwhile *M. tuberculos* is multi-resistant MJ and extensively drug resistant strain (XDR) UJ was provided by *Centre Pasteur du Cameroon.* They have been isolated from patients in the tuberculosis surveillance program of PNLT in the course of the year 2016 (*Programme National de Lutte Contre la Tuberculose*) and the sensitivity to first line and second line antibiotics was obtain using antibiogram in automatized system Bactec MGIT 960 culture. These bacterial strains were maintained on slants of both Löwenstein Jensen medium and Löwenstein Jensen medium supplemented with pyruvate (Himedia, India).

2.4.4. Preparation of Inoculum for Biological Assay

From this solid media cultures, a suspension was prepared in Middlebrook 7H9 broth lot N°0000203601 (Himedia, India), containing 0.2% v/v glycerol, 0.05% v/v tween 80 (Sigma-Aldrich, France) and 10% v/v of Middlebrook 7H9 OADC supplement *(oleic acid–albumin–dextrose–catalase*; Becton Dickinson, USA). The suspension was adjusted to turbidity compared to a 0.5 McFarland standard (10^5–10^7CFU/mL). Prior to antimycobacterial assay, the absence of contamination was confirmed by culturing in the Brain and Heart Infusion (BHI) agar medium and using Ziehl-Neelsen staining [15, 16].

2.4.5. *In vitro* Anti-mycobacterial Assessment of Essential oils

The geometric serial broth micro dilution method was carried out according to the Microplate Alamar Blue Assay (MABA) described previously by Collins and Franzblau and modified by Jimenez-Arellanes *et al.* [17, 18]. Stock solution was then added to Middlebrook 7H9 broth to reach final samples concentrations ranging from 10000 µg/mLto 156.25 µg/mL. Serial dilutions were inoculated with mycobacteria inocula (10^6 cells/mL prepared from the Middle brook 7H9 Broth) to obtained concentration ranging from 2500 µg/mL to 78.15 µg/mL. Each of the 96-wellsmicrotiter plates was mixed and incubated at 37 °C for 7 days. Positive controls consisted of Rifampicin and Isoniazid at 250 µg/mLto 7.815 µg/mL and negative controls was contained no drugs and blank contained no inoculum and nor drug. The concentration of tween 80 in the assay was maintained at a concentration to ensure that the effect on bacterial growth was minimal. Upon incubation periods, 20 µL of 0.02% resazurin salt solution were added to individual wells and the plates reincubated for one additional day and checked for colour change. Change in resazurin color from blue to pink indicated reduction of the indicator and thus bacterial growth.

The Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration of samples at which the microorganism did not demonstrate growth [19]. All the experiments were repeated three times using Isoniazid (INH) and Rifampicin (RIF) were used as antibiotic of reference.

2.4.6. Determination of anti-mycobactericidal effect

Mycobactericidal effect of essential oils and standard drugs on pathogens was determined by dilution methods [19]. The Minimal Bactericidal Concentrations (MBC) of promising essential oils was assessed by sub-culturing MIC test microtiter plates on Middlebrook broth 7H9 medium. The MBC was considered as highest dilution or lowest concentration at which no growth occurred in the medium. The anti-mycobacterial effect was deemed bactericidal or bacteriostatic depending on the ratio: MBC/MIC. Indeed, if MBC/MIC=1-2, the effect is bactericidal and if MBC/MIC=4-16, the effect is bacteriostatic [18].

3. Results & Discussion

3.1. Ethnobotanical survey

The ethnopharmacological survey included 14 respondents and yielded 12 species of plants from 07 families (Table 1). All the plant collected during the conversation with the respondents were available; most of them were found in the forest, around the home or brought from local market. Plants and plant parts mentioned by the respondents are outlined in Table 1. The results showed that most of the plant are used in association with othersfor treating tuberculosis except *Allium sativum* (bulb) *Pentadiplandra brazzeana* (roots) and *Drypetesgosseweileri* (stem barks) which usually being used alone. The most representative family of plant species were *Lamiaceae* (02); *Amaryllidaceae* (02); *Asteraceae* (02) and *Zingiberaceae* (02). A previous study carried out by Mpondo *et al.*, around eleven localities of Center region of Cameroon reported 192 plants used to treat respiratory diseases with 162 used in association with at least two plants [20]. This is probably due to the fact that, traditional treatments used by local population usually focus in the treatment of related symptoms associated to diseases. In fact, in our report we also found that *Zingiber officinale, Eucalyptus globulus, Ocimum gratissimum*, *Xylopia aethiopica* and *Allium* are also prescribed in association with other plant to cure tuberculosis and their related symptoms as report by Betti and Mpondo *et al.* [20, 21]. We also found during the conversation that these same plants could also treat respiratory diseases related to tuberculosis such as cough and lung infections.

A.sativum and *A. cepa* (bulb), *E. globulus* (leaves), *A.daniellii* (seeds) and *Z. officinale* (rhizomes) were the meanwhile the main way of recipe for preparing and administering herbal remedies was decoctions or macerations for oral rout.

Table 1: The ethnobotanical survey of medicinal plants used to treat tuberculosis in two localities of Nkam divisions

Family names	Plant samples	Part of plant and identification codes	Common or Local names	Other local use	Recipe and admitration route	Previous screened activity	Number of plant citation
Amaryllidaceae	*Allium cepa* L.	Bulbs 25742/SRF	Ndjanga	Cough; lung diseases and spice	Decoction associate with *zingiber, Allium sativum* for oral route	Anti-inflammatory antioxidant activities of essential oils [22]	6
	*Allium sativum*L.	Bulbs 44810/HNC	Ail or Garlic	Lung diseases such as pneumonias, broncho pulmonary and spice	Maceration when alone or decoction when associate with *zingiber* for oral route	Antioxidant, anti-inflammatory activities of essential oils [22]	11
Annonaceae	*Xylopia aethiopica* (Dunal) A. Rich.	Seeds of fruits 59700/HNC	Ethiopian pepper	Treatment of cough, bronchitis; spice	Decoction with *Echinops giganteus* plant for oral route	Antibacterial activity of essential oil [10] antioxidant effects of aqueous extract [23].	3
Asteraceae	*Echinops giganteus* A. Rich.	Rhizomes 23647/ SRF/CAM	Giant Japanese	Calm stomach ache and reduces asthma attacks spice.	Decoction associated with *Xylopia aethiopica* for oral route	Cytotoxicity of rhizome crude methanol extract [24]; antilavicidal activity of essential oils [25]	5
	Ageratum conizoides Line	Leaves 9503/SRF/CAM	Rois des herbes	Cough, stomach acheand wounds	Maceration or associated with *Aframamum danielli* and *Allium sativum* plant for oral route	Antibacterial and anti-germinative effect of essential oil [26]	1
Pentadiplandraceae	*Pentadiplandra brazzeana* Baill.	Roots 42918/HNC	Mgbandick	Aphrodisiac; spice; symptom of lung diseases	Used alone through decoction for oral route	Antimycobacterial activity of Methanolicextract against *M. tuberculosis* [8]; antifungal activity of essential oil [27]	5
Putranjivaceae	*Drypetesgosseweileri*S. Moore	Stem barks 25749/SRF/CAM	Nkot	Treat diarrhea; lung infections	Used alone through decoction for oral route	Antioxidant, anti-inflammatory [22] and anti-bacterial, germinative effects of essential oil [26]	3
Myrtaceae	*Eucalyptus globulu s* Labill.	Leaves 4077/SRFK	Eucaluptus	Cough; brochonpulmonary attacks	Used with *Zingiber* and *Allium sativum* in decoction for oral route	Antibacterial effect of the essential oils [10]	7
Lamiaceae	*Ocimum gratissimum* Line.	Leaves 5817/SRF/CAM	Massep	Head ache; vaginitis bronchitis and lung infections	Decoction with *Eucalyptus* and*Zingiber* for oral and inhalation route	Antibacterial, anti-germinative effects of essential oil [26]	4
	Thymus vulgaris Line.	Aerial part 25746/SRF/CAM	Thyme	Cough;bronchopulmonary disorders; spice	Infusion with *Ocimum gratisimum, Zingiber* and *Aframamum danielli* for oral route and inhalation	Antibacterial and anti-germinative effects of essential oils [10, 26]	2
Zingiberaceae	*Aframomum danielli* Hook.f	Fruits 43130/HNC	Ndong	Spice; antipoison reduced alcohol effect and pnemonia	Infusion with *Ocimum gratisimum, Zingiber*and *Thymus vulgaris* for oral route	Antilarvicidal activities of essential oil [25].	6
	Zingiber officinale Roscoe.	Rhizome 43125/HNC	Ndjinger	Cough;bronchopulmonary infection	Maceration, with *Ocimum gratisimum* and *Eucalyptus globulus* for oral route	Antibacterial and anti-germinative effects of essential oil [26]	13

3.2. Extraction Yields of the Essential oils.

The essential oils extraction from *A. sativum* bulbs produced the highest yield (0.2%) compared to those extracted from *D. gossweileri* stem-barks and *P. brazzeana* roots which yields 0.13% and 0.04% respectively. The difference between the extraction yields could be due to the impact of site and period of harvest. Nyegue *et al.* demonstrated that the extraction yield of essential oils of *P. brazzeana*, obtained by hydrodistillation can influenced by the hydrodistillation time and hydrolysis mechanisms involving chemical skeleton of the genine, the pH and ascorbates level in the environmental conditions [28]. Montaut *et al.*, 2017 showed that the quantityof glucosinolates present in plant are broken down during hydrodistillation to give the ITC compounds of entire essential oil. These glucosinolates can vary in plants and influence the extraction yields of essential oils according to some factors such as vegetative cycle of plants, their genetic and soil environment [29]. The bulbs of *A. sativum* gave a yield of 0.2% which was similar to those found in previous studies conducted by Mnayer *et al.*, but higher than those found by Khadri *et al.*, (0.4%); Lawrence and Lawrence (0.09%) [30, 31, 32]. The difference between extraction yields would be due to the impact of site and period of harvest, duration of hydrodistillation and conditions of the plant materials. The soil type, vegetative cycle of plants and climate are the factors that can also influenced the extraction yields of essential oils.

3.3. Chemical Composition of Essential oils.

The results from the chemical analysis of essential oils of *P. brazzeana* roots, *D. gossweileri* stem barks and *A. sativum* bulbs are presented in Table 2. This table showed aromatic compounds correlated with their relative percentage. The results of our investigation showed that *P. brazzeana* essential oil contained nine compounds representing 99.9% of the total essential oil where benzylisothiocyanate (91.3%) was the main compound as described by Ndoye *et al.* but at different percentage [22]. Analysis of the chemical compositions of the essential oil of *D. gossweileri* stem barks revealed five compounds representing 99.7% of the total essential oil and benzylisothiocyanate (96.0%) was the main componentas in *P. brazzeana* essential oil. Similarly, the essential oils obtained from *D. gossweileri* shoots harvested in the Central Africa Republic and Gabon were analysed by Mvé-Mba *et al.* it appears from this work that their chemical composition is particularly simple, with only three constituents: benzyl isothiocyanate representing an among of 54 to 94% and accompanied by benzyl cyanide and benzylaldehyde [33]. Instead of benzyl isothiocyanate, p-methoxybenzyl derivatives were present in *P. brazzeana* essential oil and absent in *D. gossweileri* indicating the presence of a lower quantity of p-methoxybenzylglucosinalate of which according to Nyegue *et al.*, and Montaut*et al.*, isbroken down during hydroditillation to give these derivates [28, 29]. *P. brazzeana* roots and *D. gossweileri* stem barks have in common a higher percentage of glucosinolate type compounds (benzyl glucosinolate) which justified the presence of benzyl isothiocyanate as common main derivate component in their essential oils [29]. Even if many studies confirmed benzylisothiocyanate at 99.7% as the main compounds of *P. brazzeana* roots essential oils, Nyegue in 2006 has reported that from the two samples of *P. brazzeana* roots harvested at Nsimeyong and Mount Eloundem (centre region of Cameroon) 4-methoxybenzylcyanide (47.7%) and benzylcyanide (55.0%) respectively were identified in their essential oils [27]. This can be explained in part that 4-methoxybenzylcyanide and benzylcyanide from the degradation of 4-methoxybenzyl glucosinolate and benzyl glucosinolate respectively reason why present in higher proportion in these two samples. Sometimes in aqueous media during the hydrodistillation at different pH, there is a high instability of arylaliphaticisothiocyanate compounds which can yield to these two compounds as reported by Nyegue *et al.* and De Nicola *et al.* describe [28, 34].

Concerning *A. sativum* bulbs essential oil, 22 components were identified, representing 99.4% of the total essential oil. The five major components of this oil were 2-methylpropenyl trisulfide (51.0%), allyl methyl trisulfide (12.8%), dimethyl trisulfide (11.1%), diallyl trisulfide (8.4%) and allylpropyl disulfide (5.0%). The chemical profile of essential oil from *A. sativum* bulbs showed 96.6% of allyl derivatives showing an accordance with results obtained by Mnayer *et al.*, and Ndoyé*et al.*, but where diallyldisulfide and diallyltrisulfide represented 38.0% and41.7% respectively [22, 30].The variation of major compounds found in essential oil from *A. sativum* could depend to the geographical origin, type of climate and soil as Table 2 shown. Moreover, Satyal *et al.* put in evidence that the chemical profile of *A. sativum* essential oil could have slide modifications depending to the process by which it is extracted [35]. In fact, the GC-MS analysis of essential oils of *A. sativum* bulbs extracted by three different process showed the majority presence of diallyltrisulfide ranging from 10.3 to 2.8%, diallyl disulphide 12.2 to 4.4%, allyl methyl trisulfide 13.2 to 7.9% and allyl (E) -1- propenyldisulifde 11.6 to 7.9% [35].

Table 2: Major components of *A. sativum* essential oil according to its geographical origin

Compounds (%)	Geographical origin					
	Spain [36]	**Egypt [37]**	**Serbia [38]**	**Tunisia [39]**	**France [30]**	**Greece [40]**
Diallyl sulfide	-	-	-	-	6.6	-
Diallyl disulfide	20.8	25.2	28.1-49.1	49.1	37.9	23.1–28.4
Diallyltrisulfide	33.4	21.1	30.4-33.6	30.4	28.1	18.2–22.1
Diallyltetrasulfide	-	-	-	-	4.1	-
Allyl methyl disulfide	4.4	-	-	-	3.7	8.5–11.2
Allyl methyl trisulfide	19.2	23.8	17.8	-	7.3	16.3–17.5
Allyl (E)-1-propenyl disulfide	5.2	-	-	-	-	-

Table 4: Relative percentages of constituents of essential oils from *D. gossweileri* stem-barks, *P. brazzeana* rootsand *A. sativum* bulbs.

N°	Components	LRI		Relative percentage (%)			Identification methods
			LRI	***P. brazzeana* roots**	***D. gossweileri* stem barks**	***A. sativum* bulbs**	
1	1, 2-Dithiolane	821	_	–	–	0.2	GC,RI
2	Diallylsulphide	862	_	–	–	0.2	GC,RI
3	Methylpropyl disulfide	927	_	–	–	0.1	GC,RI
4	Benzaldehyde	948	960	0.6	0.2	–	GC,RI,MS
5	Diallylsulphide	981	_	–	–	11.1	GC,RI
6	(E)-1-Allyl-2-prop-1- en-1-yl sulfide	996	_	–	–	0.3	GC,RI
7	Allyl methyl disulfide	1001	_	–	–	0.6	GC,RI
8	Methyl propyl disulfide	1040	_	–	–	5.0	GC,RI,MS
9	4-Memethyl-1,2,3-thiolane	1057	_	–	–	0.4	GC,RI,MS
10	Allyl (E)-1- propenyldisulfide	1112	_	–	–	0.5	GC,RI
11	Benzylcyanide	1121	1138	3.6	3.1	–	GC,RI
12	p-Methoxybenzaldehyde	1151	_	0.2	–	–	GC,RI,MS
13	p-Methoxylbenzylicalcohol	1163	_	0.1	–	–	GC,RI,MS
14	2-Methylpropenyl trisulfide	1202	_	–	–	51.0	GC,RI
15	2-Venyl-4H-1,3-dithiine	1210	_	–	–	0.4	GC,RI
16	Methylallyltrisulfide	1261	_	–	–	12.8	GC,RI
17	Diallyltrisulfide	1279	_	–	–	1.0	GC,RI
18	Benzylisothiocyanate	1326	_	91.3	96.0	–	GC,RI,MS
19	p-Methoxybenzylcyanide	1334	_	0.1	–	0.9	GC,RI
20	p-Methoxyphenylacetone	1380	_	0.1	–	–	GC,RI
21	5-Methyl-1, 2, 3, 4-tetrathiane	1402	_	–	–	2.2	GC,RI
22	Triallyldisulphide	1441	_	–	–	8.4	GC,RI
23	p-Methoxybenzylisothiocyanate	1515	_	3.3	–	–	GC,RI
24	1,4-Dihydro-2,3-benzoxathium-3-oxide	1573	_	–	–	0.2	GC,RI
25	Propylallylsulphide	1642	_	–	–	1.3	GC,RI
26	2-propenyl tetrasulfide	1649	_	–	–	0.7	GC,RI
27	Diallyltetrasulfide	1701	_	–	–	1.3	GC,RI
28	4-methyl-1, 2, 3, 5, 6-plutathiopane	1805	_	–	–	0.2	GC,RI
29	Dibenzylsulphide	1818	_	–	0.2	–	GC,RI
30	Benzophenone	1845	_	0.2	–	–	GC,RI
31	Methyl linolenate	1869	_	–	–	0.2	GC,RI
32	Cylooctasulfur	2004	_	–	0.6	–	GC,RI
	Total of compounds			99.9	99.7	99.4	

N°: elution order given on apolar column (HP-5); LRI: Linear Retention Index on apolar (HP-5) column; Identification methods: GC, identification based on co-injection with authentic sample; RI: Retention Indices; MS: identification based on comparison of mass spectrum withliterature data; %: relative percentage; (−): not found.

3.3. Antimycobacterial Assay

The essential oils from *P. brazzeana*, *D. gossweileri* and *A. sativum* were evaluated against four resistant strains of *M. tuberculosis*. The results presented on Table 5which showed anti-mycobacterial activity against resistant strains *M. tuberculosis*is not significant as far as each strain is concern with MICs ranging from 156.25 to 2500 µg/mL on AC45 and AC79; 78.12 to >5000µg/mL on UJ [42]. Out of these essential oils, *D.gossweileri* Exhibited its higher anti-tuberculosis activities on both the Isoniazid and Rifampicin resistant strains with MICs values of 156.25 and 1250 µg/mL respectively. The essential oil obtained from *P. brazzeana* roots and *A. sativum*, exhibited its higher anti-mycobacterial activity against the UJ strainwith MIC of 312.5 µg/mL and 78.125 µg/mLrespectively. Base on this result, these activities justified by the presence of antibacterial compounds in each essential oil such as isothiocyanate compounds and allylsulfide compounds.

In fact, some study report that synthetics isothiocyanate compounds (ITC) exhibited significant activity against H37Rv *M. tuberculosis* and three resistant strains with the MIC ranging from 0.5 to 32 µg/mL [42]. Voundi *et al.*, put in evidenceoil the inhibitory effet of *D. gossweileri* essential against four species of *Bacillus* and reported MIC ranging from 0.4 to 9.7µg/mL and anti-germinative effect against all these *Bacillus* at 2 µg/mL [26]. Even though the mechanism by which the ITC inhibits mycobacterial growth is still unknown, we can attribute the mycobacterial inhibition of the essential oil from *P. brazzeana* and *D. gossweileri* to compounds such as benzyl isothiocyanate, benzylcyanide and p-methoxybenzylisothiocyanate.It has been suggested that isothiocyanate groupscan covalently cross-link to a cellular target [43]. The isothiocyanate derivatives have a very strong antimicrobial effect due to the R-N=C=S group in the molecule. This group has a high electrophilic central carbon, which can easily react with nucleophilic centers. Furthermore, it could cleave the disulfide bonds of proteins and attack free amino acids by oxidative reaction [44, 45].

Concerning the *A. sativum* essential oil, the results obtained showed a weak activity against MDR (MJ); AC45 and AC79 isolates with MIC of 5000µg/mL and 2500 µg/mL respectively with significant activities against XDR (UJ) with MIC of 78.12 µg/mL. *A. sativum* essential oil showed higher activity than those reported by Viswanathan *et al.*,in a previous study against *M. tuberculosis* H_{37}Rv, with a reduction of 97.40% of colony at 80000 µg/mL [46]. The inhibitory effect observe could be attribute to diallyl disulfide; diallyltrisulfide and their synergistic interaction between the compounds within the essential oil. Organo sulfur compounds act by inducing membrane protein and lipid denaturation. The lipophilic properties of these compounds showed their ability to cross through the wall membrane of mycobacteria, induce

inhibition of DNA replication and perturbation of membrane proton motive force, loss of energy substrate (glucose, ATP) leading directly to death by lysis of bacteria [47]. Another mechanism attributed to the synergistic effects of the major compounds and those represented in trace amounts is action could be the inhibition of amylase and protease production, which the toxin production by the bacteria, electron flow and result in coagulation of the bacterial cell content [48].

Table 3: Plant materials used for anti-mycobacterial activity, MICs and MBCs values of their essential oil, against resistant strains of *Mycobacterium tuberculosis* AC_{45}, AC_{79}, MJ and UJ.

Essential oils	Inhibition parameters (µg/mL)	AC_{45}	AC_{79}	MJ	UJ
D. gossweileri	MIC	156.25	1250	>5000	>5000
	MBC	2500	>5000	n.d	n.d
	MBC/ MIC	16	n.d	n.d	n.d
P. brazzeana	MIC	625	1250	2500	312.50
	MBC	2500	2500	5000	625
	MBC/ MIC	4	2	2	2
A. sativum	MIC	2500	2500	5000	78.12
	MBC	2500	>5000	>5000	312.50
	MBC/ MIC	1	n.d	n.d	4
Rifampicin	MIC	0.97	>1000	>1000	>1000
	MBC	7.81	n.d	n.d	n.d
	MBC/ MIC	8	n.d	n.d	n.d
Isoniazid	MIC	n.d	3.90	n d	n d
	MBC	n.d	15.625	n.d	n.d
	MBC/ MIC	n.d	4	n.d	n.d

MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; >5000: not showing inhibition even at the highest test concentration of 5000 µg/mL; n.d: not determined;AC_{45}: Isoniazid resistant*M. tuberculosis* strain; AC_{79}: Rifampicin resistant*M. tuberculosis* strains; MJ: MDR *M. tuberculosis* strain and UJ: XDR *M. tuberculosis* strain.

4. Conclusion

The emergence and spread of multi-drugs resistant strains around the world making the control of tuberculosis difficult and looking for alternative or complementary as plant components solution could ameliorate this situation. *D. gossweileri*,*P. brazzeana*and *A. sativum* used by the population of Nkam division to treat tuberculosis showedits anti-mycobacterial potential. The results obtained in this study can serve as a preliminary baseresults for ethnopharmacological research about used medicinal plant and their *in vitro* activity. Therefore, more attention should be given to research about the efficacy of Cameroonian aromatic and medicinal plants against the complex *Mycobacterium tuberculos* is which could yield to information improvising the existence of this therapeutic practices.

5. Appendix

GC:Gas Chromatography; GC/MS:Gas Chromatography coupled to Mass Spectrometry; HIV: Human Immunodeficiency Virus; HBV: Human B Virus; HCV: Human C Virus; INH: Isoniazid; MABA: MicroplateAlamar Blue Assay; MBC: Minimal Bactericidal Concentrations; MDR: Multi Drug Resistant; MIC: Minimum Inhibitory Concentration; OADC: *Oleic acid–Albumin–Dextrose–Catalase*; RIF: Rifampicin; TB: Tuberculosis; XDR: Extensively Drug Resistant; WHO: World Health Organization.

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