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Antimicrobial potentials of essential oils extracted from West African aromatic plants on common skin infections☆,☆☆

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ABSTRACT

During the last decade, the advent of multi-drug resistant pathogens responsible for skin infections tends to make conventional treatments obsolete. Even though many studies have reported the antimicrobial properties of essential oils (EOs), the inconsistent use of various susceptibility testing methods has made information on antimicrobial potential of many EO varieties fragmentary. Using a single method approach, the objective of this work was to assess and to compare the antibacterial and antifungal properties, against skin pathogens, of EOs extracted from West African aromatic plants.

Twenty-three plant samples collected in Benin and Burkina Faso were screened against 20 bacterial and fungal isolates obtained from skin lesions. Activity was evaluated by the determination of minimal inhibitory concentrations (MICs), with readings facilitated by the use of resazurin, a blue dye metabolized into pink resorufin by viable cells.

Following this screening, nine EOs were found particularly active with MICs lower than 0.35% v/v. Gas Chromatography-Mass Spectrometry (GC/MS) analysis was used to determine the phytochemical profile of these active EOs which were found exceptionally rich in oxygenated monoterpenes, especially aldehydes, alcohols or phenols and their derivatives.

Through this study, we demonstrated that several West African EOs have a significant antimicrobial potential which could, however, be considerably impacted by plant growing or harvesting place due to phytochemical composition variation. These EOs, even if their antimicrobial effects appeared lower than those of conventional antibiotics, constitute eas-

Abbreviations: EO, Essential oil; MIC, Minimum Inhibitory Concentration; GC/MS, Gas Chromatography coupled with Mass Spectrometry; CLSI, Clinical and Laboratory Standards Institute; CA-SFM, Comité de l'antibiogramme. French Society of Microbiology; EUCAST, European Committee on Antimicrobial susceptibility testing.

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ily available mixtures of active compounds and could nevertheless be considered, in the context of increasing multidrug resistance, as complementary or alternative therapies in common skin infections management.

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1. Introduction

Skin infections are caused by bacteria, fungi, viruses and parasites. Bacterial and fungal skin infections, in particular, are commonly found in tropical regions because of the ambient temperature and humidity sometimes associated with poor hygiene, and are present in various clinical forms [1]. Indeed, skin is an ideal environment for microbial growth because of its abundant supply of nutrients, water and high temperature. As a result, it is normally covered with various microorganisms. Bacterial or fungal infection occurs when the balance between the host and the microorganisms is altered.

The importance of skin diseases is often trivialized in sub-Saharan Africa; as they are usually not fatal, they tend to be ignored [1]. For example, dermatological conditions accounted for only 1.5% of the diseases reported in hospitals in Benin in 2017 and 2.1% of external consultation reasons in primary healthcare units in Burkina Faso in 2018 [2,3]. However, in tropical regions, the prevalence of dermatoses is high. Specific data are not available for Benin and Burkina Faso, but data from other West-African countries (Nigeria, Ghana, Ivory Coast) show that it ranges from 21 to 87% [4–7]. The frequency of skin infections has risen sharply with the development of Acquired Immunodeficiency Syndrome (AIDS). During human immunodeficiency virus (HIV) infection, cutaneous and mucosal manifestations are present at all stages, from primary infection to the onset of AIDS. For example, in Cotonou (Benin), a retrospective transverse study, conducted in 2012 on 152 analyzed files of HIV-positive patients, identified 274 cutaneous and mucosal manifestations [8]. Skin infections related to HIV/AIDS or their treatments have become increasingly common and are reported to occur in up to 95% of patients. They may be indicative of the disease and may even be of prognostic interest.

The advent of multidrug-resistant bacteria in dermatology [9,10], with recurrent therapeutic failures as a corollary, is a situation that considerably aggravates the problem associated with these infections. Beyond the physical health problem, skin infections, due to their unsightly appearance, have an important psychosocial repercussion. Indeed the skin has an eminent place in the edification of the personality and especially of the psychic life. Faced with this situation, some authors believe that essential oils (EOs), already widely used in skin aromatherapy, could, as a result of their antimicrobial properties, constitute valid alternatives to antibiotics and antifungals. Indeed, as they are mixtures of compounds which can affect different targets, they can hardly be subjected to microbial resistance [11].

The aromatherapy literature identifies numerous EOs for dermatological use, most of which are recommended for infections and more than 100 are cited in reviews for the general public [11]. In this context, EOs are mainly used for the treatment of infections caused by bacteria and fungi. Indeed, Orchard and Van Vuuren in 2017, reviewed 98 EOs recommended for dermatological use among which 73 are used for treatment of bacterial infections, 34 for treatment of fungal infections and 16 for treatment of viral infections [11].

Although there are many studies evaluating the antimicrobial properties of EOs, the wide variability in evaluation methods from one study to another does not allow an easy comparison of activities to identify the most effective oils. Indeed, different methods are used, namely tube dilution method [12,13], broth microdilution method [14], agar dilution method, disc diffusion method and vapor contact method . Certain methods widely used in the literature, in particular the diffusion tests, are more of pre-screening than a real evaluation because they do not take into account the real activity of EOs which, because of their hydrophobicity, have a low diffusibility in agar medium. Moreover, the microbial strains used are very variable from one study to another with very different or even unknown resistance profiles, which does not favor comparison either.

Hence the aim of the study was to determine the antimicrobial potential of EOs extracted from medicinal plants collected from Benin and Burkina Faso against bacteria and fungi implicated in skin infections in West Africa.

2. Materials and Methods

2.1. Plant Materials And EOS Extraction

The plant materials were aromatic plants of Beninese and Burkinabe origin. Indeed, these two countries put together are representative of the 4 main agro-ecological zones of West Africa, namely Sahelo-Sudanian, Sudano-Guinean, Guinean and forest zones (Fig. 1) [15].

Given the plethoric number of aromatic plants in these two countries, plants were selected based on mention in scientific literature of antimicrobial activity of their EOs and/or an empirical use of those plants by local populations in the

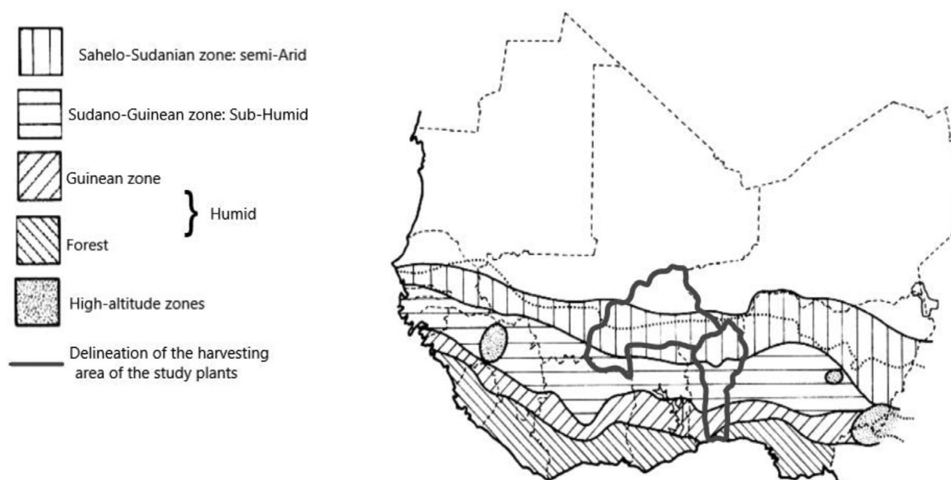

Fig. 1. Map of West Africa agro-ecological zones showing delineation of harvesting area of plants used for EOs extraction (Adapted from Jahnke, 1982) [15]

management of skin conditions. Twenty-three (23) plant specimen were harvested in Benin (from October 2014 to February 2015) and Burkina Faso (from November 2014 to June 2015) and were authenticated according to harvesting place by the National Herbarium of Benin or Burkina Faso where voucher specimen were deposited. Selected plants, organs used for EOs extraction, collection place and period, and voucher specimen number are presented in Table 1.

Prior to extraction, plant materials were air-dried under shade in an air-conditioned room at a temperature of 20 °C ± 4 °C for three weeks. The EOs were then extracted by hydrodistillation from 200 g of air-dried material for 3 h using a Clevenger-type apparatus. The extraction yields were determined as the percentage w/w of the EO quantity according to mass of air-dried plant material. The extracted EOs were stored at 4 °C in sealed brown glass vials prior to use.

2.2. GC/MS analysis of EOs

The GC/MS analyses were performed using a TRACE GC 2000 series apparatus (Thermo Quest, Rodano, Italy) interfaced with a TRACE MS (Thermo Quest) operating in electron impact mode at 70 eV and equipped with a CP-Wax 52CB column (25 m x 0.25 mm, 0.2 µm film thickness, Chrompack). Helium was used as carrier gas at a constant flow rate of 1.3 mL min^{-1} and oven temperature was programmed at 65 °C for 5 min and then increased to 185 °C at a rate of 2 °C min^{-1} followed by a final increase (3 °C min^{-1}) to 230 °C which was maintained for 10 min. Using an autosampler (AS2000, Thermo Quest), 1 µL of EO at 1% v/v in TBME was injected in GC/MS (front inlet temperature fixed at 250 °C, GC/MS interface temperature at 260 °C). Chromatographic and spectral data were recorded and analyzed using Xcalibur 1.1 software. For compounds identification, mass spectra of resulting peaks were compared with the NIST/ EPA/NIH 98 library and identification was declared valid for direct and reverse match factors (SI and RSI) greater than 900. Quantitative values were obtained from GC-MS data using the normalization procedure.

2.3. Antimicrobial Screening of EOs

The antimicrobial screening of EOs was performed by determining minimal inhibitory concentrations (MICs) which represent the lowest EO concentrations that prevent visible growth of the concerned microorganisms in the appropriate medium [16].

2.3.1. Strains

All the EOs were tested on four reference strains, two strains of *Staphylococcus aureus* (a methicillin-susceptible one: ATCC 25923, and a methicillin-resistant one: ATCC 33591), and two strains of *Pseudomonas aeruginosa* (a wild-type strain: PAO1 and another one deleted for efflux pump expression: PAO509). Following this prescreening, the most effective EOs were then tested on five clinical strains of bacteria and 14 clinical strains of yeasts involved in common skin infections in Benin. These clinical strains were selected from the collection of the Hubert Koutoukou Maga University Hospital (CNHU-HKM) in Cotonou. The reference and clinical strains tested are listed in Table 2 where their characteristics and clinical sampling fields are specified.

Bacterial strains were stored at -80 °C in a sterile solution of cation-adjusted Mueller-Hinton broth (caMHB, Becton, Dickinson and Company, Franklin Lakes, NJ) supplemented with 20% glycerol [17]. Yeast strains were also stored at -80 °C in a sterile solution of Sabouraud-dextrose broth (Sabouraud Dextrose Liquid Medium, Oxoid, Basingstoke Hampshire, United Kingdom) supplemented with 20% glycerol [17].

Table 1
Scientific names of selected plants, studied organs, identification number

Families	Species	Collection location _Period (*Season*)[a]	Part of plant collected	Voucher specimen Number
Poaceae	*Cymbopogon citratus* (DC.) Stapf	Benin (Cotonou)_10/2014(*WS*)	Leaves	AA 6635/HNB
		Burkina Faso (Ouagadougou)_04/2015(*DS*)		8731
	Cymbopogon nardus (L.) Rendle	Benin (Cotonou)_10/2014(*WS*)	Leaves	AA 6637/HNB
		Burkina Faso (Ouagadougou)_11/2014(*DS*)		8729
	Cymbopogon giganteus (Hochst.) Chiov.	Benin (Dassa)_02/2015(*DS*)	Leaves	AA 6636/HNB
		Burkina Faso (Koupela)_01/2015(*DS*)		8733
	Cymbopogon schoenanthus (L.) Spreng	Burkina Faso (Zempassogo)_11/2014(*DS*)	Leaves	8732
Lamiaceae	*Ocimum gratissimum* (L.)	Benin (Cotonou)_10/2014(*WS*)	Stems and leaves	AA 6634/HNB
		Burkina Faso (Ouagadougou)_05/2015(*WS*)[*]		8736
	Ocimum canum Sims; (*Ocimum americanum*)	Benin (Porto-Novo)_12/2014(*DS*)	Stems and leaves	AA 6633/HNB
		Burkina Faso (Ouagadougou)_11/2014(*DS*)		8734
	Ocimum basilicum (L.)	Burkina Faso (Ouagadougou)_12/2014(*DS*)	Stems and leaves	8730
	Hyptis suaveolens (L.) Poit.	Benin North (Parakou)_02/2015(*DS*)	Stems and leaves	AA 6632/HNB
		Benin South (Porto-Novo)_12/2014(*DS*)		AA 6632/HNB
	Mentha piperita Huds. L.	Burkina Faso (Ouagadougou)_05/2015(*WS*)[*]	Stems and leaves	8727
Verbenaceae	*Lippia multiflora* Moldenke	Benin (Porto-Novo)_02/215(*DS*)	Leaves	AA 6629/HNB
		Burkina Faso (Ouagadougou)_11/2014(*DS*)		8728
	Lantana camara L.	Burkina Faso (Kamboinse)_06/2015(*WS*)	Leaves	8735
Myrtaceae	*Eucalyptus camaldulensis* Dehn.	Benin (Cotonou)_ 02/2015(*DS*)	Leaves	AA 6630/HNB
	Eucalyptus citriodora Hook	Benin (Abomey-Calavi)_12/2014(*DS*)	Leaves	AA 6631/HNB
Rutaceae	*Clausena anisata* (Willd.) Hook	Benin (Bohicon)_ 02/2015(*DS*)	Leaves	AA 6638/HNB
Zingiberaceae	*Curcuma longa*	Benin (Porto-Novo)_ 02/2015(*DS*)	Rhizome	AA 6640/HNB
Chenopodiaceae	*Chenopodium ambrosioides* L.	Benin (Cotonou)_ 02/2015(*DS*)	Leaves	AA 6628/HNB

[a] Collection Period is expressed as month/year and season as *DS*, Dry Season and *WS*, Wet Season
[*] Start of Wet Season

Table 2
Reference bacterial and clinical microbial isolated strains used during EO antimicrobial susceptibility testing

	Strains	Characteristics	Origin
Bacterial Reference Strains	*Staphylococcus aureus* (MSSA ATCC 25923)	Sensitive to β-lactams	American Type Culture collection
	Staphylococcus aureus (MRSA ATCC 33591)	Resistant to β-lactams by production of PBP2a and β-lactamases	American Type Culture collection
	Pseudomonas aeruginosa PAO1	Wild type	American Type Culture collection
	Pseudomonas aeruginosa PAΔpump (PAO509)	PAO1 deleted for expression of MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY-OprM efflux pumps	Louvain Drug Research Institute
Bacterial Clinical Strains	*Staphylococcus aureus*	Skin and underlying tissues infection	CNHU-HKM Collection
	Staphylococcus epidermidis	Skin and underlying tissues infection	CNHU-HKM Collection
	Acinetobacter baumannii	Skin and underlying tissues infection	CNHU-HKM Collection
	Pseudomonas aeruginosa	Skin and underlying tissues infection	CNHU-HKM Collection
	Streptococcus pyogenes	Skin and underlying tissues infection	CNHU-HKM Collection
Yeast Clinical Strains[a]	*Candida albicans* (YS-04)	Onychomycosis (Male 64 yrs)	CNHU-HKM Collection
	Candida albicans (YS-05)	Intertrigo (Female 30 yrs)	CNHU-HKM Collection
	Candida albicans (YS-07)	Onychomycosis (Female)	CNHU-HKM Collection
	Candida albicans (YS-61)	Athlete's foot (Male 60 yrs)	CNHU-HKM Collection
	Candida albicans (YS-78)	Dermatosis	CNHU-HKM Collection
	Candida albicans (YS-93)	Vulvo-vaginal infection (Female)	CNHU-HKM Collection
	Candida parapsilosis (YS-08)	Athlete's foot (Male 65 yrs)	CNHU-HKM Collection
	Candida parapsilosis (YS-09)	Onychomycosis	CNHU-HKM Collection
	Candida parapsilosis (YS-26)	Onychomycosis	CNHU-HKM Collection
	Candida krusei (YS-68)	Dermatosis	CNHU-HKM Collection
	Candida tropicalis (YS-151)	Dermatosis	CNHU-HKM Collection
	Candida guilliermondii (YS-119)	Candidemia	CNHU-HKM Collection
	Candida haemuloni (YS-133)	Dermatosis (sea fisherman)	CNHU-HKM Collection
	Trichosporon cutaneum (YS-98)	Dermatosis (Male 41 yrs)	CNHU-HKM Collection

[a] Yeast strain ID (YS) in CNHU/HKM collection is put in bracket.

Table 3
Antibiotic resistance profile of bacterial clinical strains determined by disc diffusion

Bacterial strains	Resistance profiles obtained[a]
Staphylococcus aureus	PEN G-R; GEN-R; TOB-R; KAN-R; TET-R, NET-R
Staphylococcus epidermidis	PEN-R; GEN-R; TOB-R; KAN-R; CHL-R; SXT-R, FOX-R
Acinetobacter baumannii	IPM-R; CIP-R; GEN-R; TOB-R; NET-R; CHL-R; TET-R; SXT-R
Pseudomonas aeruginosa	GEN-R; NET-R
Streptococcus pyogenes	PEN G-R, TET-R; ERY-R; SXT-R

[a] Only relevant resistance profiles were considered in this table. R, Resistant; PEN, Penicillin; PEN G, Penicillin G; GEN, Gentamicin; TOB, Tobramycin; KAN, Kanamycin; TET, Tetracyclin; NET, Netilmicin; CHL, Chloramphenicol; SXT, trimethoprim-sulfamethoxazole; IPM, Imipenem; CIP, Ciprofloxacin; ERY, Erythromycin; FOX, Cefoxitin.

2.3.2. Antibiotics Standards

Four commercial antibiotics were used as microbiological standards for test control when determining MICs of EOs on reference strains: oxacillin, ampicillin and norfloxacin from Sigma-Aldrich (St Louis MO, USA); and moxifloxacin HCl from Bayer (Leverkusen, Germany). These antibiotics were selected because of their characteristic MIC values ([16]) on the different reference strains in order to serve both as quality control of the strains and of the manipulations carried out during MIC determination. In addition, they served as a reference in order to compare the activity of EOs with those of conventional antibiotics to which the strains are sensitive or resistant.

Futhermore, since only fragmented information was available on gravity of skin lesions where clinical bacterial strains were isolated, their antibiotic resistance profiles was determined through an antibiotic susceptibility testing (AST). AST was performed by disc diffusion method according to CASFM/EUCAST 2018 guidelines [18]. The following antibiotic discs (drug concentration in µg) all provided by Bio-Rad Laboratories (Marnes-la-Coquette, France) were tested: Penicillin G (6 µg), Ampicillin (10 µg), Amoxicillin (25 µg), Amoxicillin/Clavulanic Acid (20/10 µg), Oxacillin (5 µg) and Ticarcillin (75 µg) for penicillins; Cefoxitin (30 µg), Cefuroxime (30 µg), Cefotaxime (30 µg) and Ceftriaxone (30 µg) for cephalosporins; Imipenem (10 µg) for carbapenems; Aztreonam (30 µg) for monobactames; Gentamicin (10 µg), Tobramycin (10 µg), Kanamycin (30 µg) and Netilmicin (10 µg) for aminoglycosides; Norfloxacin (10 µg), Ofloxacin (5 µg), Ciprofloxacin (5 µg) and Fusidic Acid (10 µg) for quinolones; Erythromycine (15 µg) for macrolides; Tetracycline (30 µg) and Doxycyclin (30 µg) for cyclines; Lincomycin (15 µg), Pristinamycin (15 µg), Trimethoprim/Sulfamethoxazole (1.25/23.75 µg), Chloramphenicol (30 µg), Vancomycin (30 µg) and Fosfomycin (50 µg) for others. Antibiotic resistance profiles are given in Table 3.

2.3.3. Minimal Inhibitory Concentration (MIC) determination

MICs of EOs were determined by broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [19] in 96-well microplates with caMHB (for bacteria) and liquid Sabouraud (for yeasts) as culture media.

Briefly, before antimicrobial testing, bacterial strains were cultured overnight (for approximately 12–16 h) at 37 °C on TSA agar (Tryptic Soy Agar) or Mueller-Hinton agar (Himedia Laboratories, Mumbai, India) and yeast strains were cultured on Sabouraud chloramphenicol agar (Microxpress, Tulip Diagnostics Ltd, Goa, India) and incubated at 25 °C for 48 h. Suspensions of bacteria and yeasts at 0.5 McFarland were prepared respectively in caMHB and liquid Sabouraud and diluted 1/100 to obtain starting inocula at 10^6 CFU mL^{-1}.

Each EO was emulsified in the appropriate nutrient broth (caMHB for bacterial tests or liquid Sabouraud for yeast tests) using polysorbate 80 (at a concentration of 4% v/v in nutrient broth) as surfactant to obtain a concentration of EO of 4% v/v. This high concentration of polysorbate 80 (equal to EO concentration) was selected in order to obtain a very stable and thin EO emulsion not only to ensure homogeneity of the EO dispersion into nutrient broth but also to prevent volatilization during the testing [20]. Serial two-fold dilutions were then made to obtain final EO test concentrations of 1% to 0.0078% v/v after addition of microbial inocula. The test concentrations were established based on a literature review of EOs with antimicrobial potential and on the maximum empirical EO content (1–2% v/v) usually found in aromatherapy drugs. Two positive and two negative growth controls were performed for each plate. Since polysorbate 80 was also diluted in test wells at a concentration range between 1% and 0.0078% v/v, the first positive growth control was to test for microbial growth in nutrient broth supplemented only with polysorbate 80 at these final concentrations while the other control was to test for growth in nutrient broth without tween. The plates were aerobically (normal atmospheric conditions) incubated at 37 °C for 16–20 h for bacteria and at 25 °C for 48 h for yeasts. In order to prevent evaporation, the microwells were filled only to one third of their capacity and the plates were fitted with hermetic lids. A visual inspection of the turbidity at the bottom of the wells was carried out. And then, in order to facilitate the detection of microbial growth [16] and to confirm first visual inspection, 30 µL of a 0.02% w/v sterile aqueous solution of resazurin (Sigma-Aldrich, St Louis MO, USA) was added to the wells and the plates were reincubated for 60 min at 37 °C for bacteria and 120 min at 25 °C for yeasts. Microbial growth was evidenced by a reduction of blue resazurin to pink resorufin. The MIC was considered as the lowest concentration of EO for which the well did not change color from blue to pink [16]. All tests were made in triplicate.

Table 4
Essential oils extraction yields and their major components determined by GC/MS analysis

Species	Collect locat.	Yield (%)	Major components (Percentage of presence)
C. citratus	BN	1.6	geranial (47.8); neral (35.5); geraniol (4.4); sulcatone (2.7); linalool (1.7); β-pinene (1.4)
	BF	1.4	geranial (50.9); neral (45.3)
C. nardus	BN	1.2	citronellal (27.3); cis-geraniol (21.3); citronellol (8.4); elemol (6.0); menthol (3.5); τ-cadinol (2.8)
	BF	2.0	citronellal (74.9); cis-geraniol (9.6); tetrahydroionol (6.0)
C. giganteus	BN	0.4	*p*-mentha-1(7),8-dien-2-ol (39.0) ; carveol (35.1) ; carvone (3.3); cosmene (1.4)
	BF	0.5	carveol (35.1); *p*-mentha-1(7),8-dien-2-ol (30.0); cosmene (18.2); *trans*-α-bergamotene (3.6); carvone (2.3)
C. schoenanthus	BF	2.2	piperitone (38.1); 2-isopropyl-5-methyl-3-cyclohexen-1-one (28.5); elemol (9.3); γ-eudesmol (2.1)
O. gratissimum	BN	1.5	β-cymene (52.0); carvacrol (22.9); γ-terpinene (5.5); β-selinene (3.8); iso-β-caryophyllene (3.5); *p*-cymenene (3.1); *trans*-sabinene hydrate (2.4), terpinen-4-ol (1.2); α-selinene (1.1)
	BF	1.2	β-cymene (51.5); α-terpinene (33.2); carvacrol (5.3); *p*-cymenene (3.1); thymol methyl ether (2.4)
O. canum	BN	1.3	terpinen-4-ol (46.1); linalool (22.2); *trans*-sabinene hydrate (7.1); γ-terpinene (7.1); α-bergamotene (3.2); terpinolene (2.6); α-farnesene (1.1); β-cymene (1.0)
	BF	1.2	camphor (32.0); bornyl acetate (15.2); α-bergamotene (11.3); *cis*-caryophyllene (6.5); α-terpineol (6.4); β-cymene (5.5); α-farnesene (3.2); caryophyllene oxide (1.8); piperitone (1.5)
O. basilicum	BF	0.2	linalool (60.8); eugenol (7.7); α-bergamotene (7.3); γ- cadinene (3.1); α-ocimene (1.4)
H. suaveolens	BN (N)	0.4	*p*-cymene (42.3); L-fenchone (30.0); fenchol (16.7)
	BN (S)	0.4	β-caryophyllene (32.1); α-caryophyllene (11.7); *trans*-farnesol (7.9); α-terpinolene (1.3); (+)-2-bornanone (1.2)
M. piperita	BF	2.0	menthofurane (29.6); γ-terpineol (28.3); L-menthone (24.8); menthol acetate (7.7); pulegone (3.9); menthol (2.9)
L. multiflora	BN	1.0	pinocarveol (51.0); terpineol (12.5) ; linalool (7.7) ; *trans*-β-farnesene (5.3) ; caryophyllene (5.3); myrtenal (2.8); germacrene D (2.3)
	BF	2.3	γ-terpinene (23.1); β-cymene (53.2); β-cis-caryophyllene (13.6); thymol acetate (3.6); carvacrol (4.1)
L. camara	BF	0.2	isocaryophyllene (43.4); γ-terpinene (10.9); *o*-cymene (7.7)
E. camaldulensis	BN	2.1	terpinolene (36.8); β-cymene (26.4); α-terpineol (6.1); terpinen-4-ol (4.6); spathulenol (4.0); α-gurjunene (1.8)
E. citriodora	BN	2.6	citronellal (49.1); isopregol (20.4); tetrahydroionol (9.9); β-caryophyllene (2.9)
C. anisata	BN	0.8	estragole (94.8)
C. longa	BN	ND	curcumene (25.6); (S)-spiro [4.4]nona-1,6-diene (17.6); precocene I (3.0); α-terpinolene (2.6); β-cymène (2.2)
C. ambrosioides	BN	1.5	β-cymene (47.1); 5-isopropyl-6-methyl-hepta-3,5-dien-2-ol (19.2); (E)-2,3-epoxycarane (2.1)

3. Results and Discussion

3.1. GC/MS Characterization and Prescreening of EOs by Testing on Reference Bacterial Strains

Phytochemical analysis by GC/MS allowed us to determine the volatile components of studied EOs. These volatile compounds belong to several chemical families including terpenes, terpenoids and phenylpropanoids. The major components (percentage $>$ 1%) are reported in Table 4. With the exception of EOs such as *O. canum, H. suaveolens* and *L. multiflora*, which may belong to different chemotypes, the major components of EOs of the same species are often the same regardless of their geographical origin. However, as shown in Table 4, quantitative differences are observed. The minor compounds which are not included here, are however very variable from one geographical origin to another.

The chemical composition of an EO depends on many factors. In addition to the factors linked to the processing operations during extraction (factors relatively negligible in our study due to the standardization of the extraction method), the chemical composition of an EO is mainly a function of environmental factors and above all of the genetic endowment of the plant (notion of chemical race or chemotype). While the presence or absence of a chemical constituent at any stage of growth is exclusively determined by the genetic endowment of the plant, its concentration is governed by both genetic and environmental factors . The influence of climatic factors is particularly important when the EO storage structures are located on the plant surface (as it is the case with leaves, main plant organ sampled during this study) . Within the context of this climatic influence, while plants originated from temperate regions are sensitive to the length of days, plants of tropical origin are more impacted by the alternation of dry and wet seasons [21]. For example, by taking the EOs of *C. citratus* and *C. nardus*, the concentration of aldehydic monoterpenoids (major components) seems to vary with environmental parameters such as the season. Indeed the respective samples of EOs of *C. citratus* and *C. nardus* from Burkina Faso (harvested in the dry season) are richer in citral (geranial and neral) and citronellal than their Beninese counterparts harvested in the rainy (wet) season. This could be explained by the fact that, more often than not, a plant under water stress (when the environmental humidity decreases) tends to produce more secondary metabolites (e.g. terpenes in aromatic plants). Some species,

Table 5
Prescreening results on reference bacterial strains

Species	Collect location	*Staphylococcus aureus*		*Pseudomonas aeruginosa*	
		MSSA ATCC 25923	MRSA ATCC 33591	PAO1	PAO1Δpumps (PAO509)
C.	BN[*]	0.25–0.5[a]	0.25–0.5	>1	0.5
citratus	BF[*]	0.125–0.25	0.35	>1	0.5
C.	BN	0.06–0.125	0.125–0.25	>1	0.25
nardus	BF	0.125–0.25	0.125–0.25	>1	0.25
C.	BN	0.125–0.25	0.25–0.5	0.5	0.125–0.25
giganteus	BF	0.5	0.25–0.5	1	0.25
C. schoenanthus	BF	0.25–0.5	0.25–0.5	>1	>1
O.	BN	0.5–1	0.25–0.5	>1	1
gratissimum	BF	0.25–0.5	0.125–0.25	>1	0.5
O.	BN	0.5–1	0.5–1	>1	1
canum	BF	0.5–1	1	>1	>1
O. basilicum	BF	0.125–0.25	0.125–0.25	1	0.25
H.	BN (North)	0.5	0.5–1	>1	>1
suaveolens	BN (South)	1	1	>1	>1
M. piperita	BF	1	1	>1	>1
L.	BN	0.25–0.5	0.5–1	>1	0.25
multiflora	BF	0.25–0.5	0.125–0.25	>1	0.5
L. camara	BF	1	1	>1	>1
E. camaldulensis	BN	0.25–0.5	0.71	0.5	0.5
E. citriodora	BN	1	1	>1	1
C. anisata	BN	>1	>1	>1	>1
C. longa	BN	1	>1	>1	>1
C. ambrosioides	BN	1	>1	>1	0.06–0.125
Oxacillin		0.0625 [b]	32 [b]	-	-
Ampicillin		0.5 [b]	16 [b]	-	-
Norfloxacin		-	-	0.36 [b]	0.03 [b]
Moxifloxacin		0.032 [b]	0.032 [b]	0.5 [b]	0.015 [b]

[a] The MIC values of EO are expressed in % vol/vol (n = 3)
[b] The MIC values of control antibiotics are expressed in μg mL^{-1} = 0.0001% w/v
[*] BN, Benin; BF, Burkina Faso

however, experience a decrease in their capacity to produce these secondary metabolites. In fact, climatic variations impact the vegetative cycle of the plant and also its EO composition .

However, plants such as *M. piperita*, originating from temperate regions, seem to have a composition in tropical environments which is affected by day length. According to Bruneton in 2009, when *M. piperita* is subjected to long days and short nights, the menthofuran level increases and the menthol level decreases; the situation is reversed when the days get shorter. That could in part explain the difference in major components observed between the Burkinabe sample of *M. piperita* (rich in menthofuran) and Mediterranean samples (rich in menthol) [22].

All the EO samples have been tested on two reference strains of *S. aureus* (a methicillin-susceptible one and a methicillin-resistant one) and two strains of *P. aeruginosa* (a wild strain and a derivative thereof that does not express the main efflux pumps extruding antibiotics). MICs are summarized in Table 5.

Although most of the tested EOs were active on *S. aureus*, eight EOs were characterized by their lower MIC values on both methicillin-sensitive and methicillin-resistant strains of *S. aureus*, namely the EOs of *C. citratus* from Benin and Burkina Faso (BF), *C. nardus* from Benin and BF, *C. giganteus* from Benin, *O. gratissimum*, *O. basilicum* and *L. multiflora* from BF.

C. citratus EO from Benin showed identical MIC values on both strains of *S. aureus*. These values are almost equal to those obtained with *C. citratus* EO from BF, with the difference that the latter showed a slightly better activity on the methicillin-sensitive strain. These results are very close to those obtained by Inouye et al. in 2001 [23] on the FDA 209P (JC-1) methicillin-sensitive strain with a MIC of 0.17% v/v obtained by broth dilution method. These close results can be explained by the very similar phytochemical compositions between these different EOs, with neral and geranial as major compounds. However, their higher percentage in Burkinabe EO (45.3% and 50.9%) may explain the more pronounced antimicrobial activity than that of Benin (35.4% and 47.8%). These remarks are confirmed by the works of Onawunmi et al. [24] who demonstrated that the antibacterial activity of *C. citratus* EO is due to neral and geranial.

Both Beninese and Burkinabe *C. nardus* EOs showed the same activity on the methicillin-resistant strain (MRSA) of *S. aureus*. This result is similar to that (0.12% v/v) on MRSA NCTC 6571 obtained by Hammer et al. [25] by broth microdilution method. However, on the methicillin-sensitive strain (MSSA), the Beninese EO was more active than that from BF. Contrary to the conclusions of the work of Lertsattithanakorn et al. in 2006 [26], which attributed the antibacterial activity of *C. nardus* EO to citronellal because of its high concentration; it must be admitted here that we cannot draw the same conclusions. Indeed, the EO from BF, although having a higher citronellal content (74.9%) than that of Benin (27.3%), was nevertheless

found to be less active. Even if it would be imprudent to deny any involvement of citronellal in the activity, cis-geraniol (nerol), more abundant in Beninese EO, may also be responsible for this anti-staphylococci activity.

The activity of *C. giganteus* on *S. aureus* was shown by the work of Jirovetz et al. in 2007 [27] who found a much higher activity on *S. aureus* ATCC 6538P (0.006% v/v) by the agar dilution method. According to this author this activity is due to *p*-menthadienols which are found in our study in the Beninese oil at a concentration of 39% and in Burkinabe oil at a concentration of only 30%. This difference could probably explain, at least partially, the difference in activity.

O. gratissimum EO from BF was more active than that from Benin. Nakamura et al, in 1999 [12] had already demonstrated a very close activity (0.1% v/v) of an EO sample from BF by broth macrodilution method. Kpoviessi et al. [13] attributed the activity of *O. gratissimum* EO to thymol, but even it was not identified in our work, some studies revealed an enhancement of antimicrobial properties in thymol ether derivatives compared to thymol [28]. Indeed, both chemotypes contained thymol methyl ether (2.4% in Burkinabe EO and less than 1% in Beninese one) known for its antibacterial properties. The EO antimicrobial potential could also be attributed in part to the presence of other compounds such as carvacrol [29] and γ-terpinene [30].

O. basilicum EO was found to be very active on MRSA and MSSA strains. This activity is similar to that found by Hussain et al. in 2008 [31] who obtained a MIC of 0.15% w/v on the MSSA strain (ATCC 25923). According to the conclusions of Lertsatitthanakorn et al. [26], the pharmacophore is linalool, which was present here at a level of 60.8%. Indeed, according to Hussain et al. [31], linalool has a MIC on *S. aureus* of 400 μg mL^{-1} (i.e. 0.04% w/v).

The EO of *L. multiflora* from BF was more active than the one from Benin on the MRSA strain. On the other hand, these two oils had the same activity on the MSSA strain. Bassole et al. [14] in BF in 2003 had already demonstrated an activity but much higher (0.06% v/v) on MSSA ATCC 25923 by Broth Microdilution method. They had attributed the antibacterial activity of this EO mainly to linalool. In our study, this compound, present at a concentration of 7.7% in the Beninese EO could in part explain its activity. However, as phytochemical analysis of the Burkina Faso species did not reveal the presence of linalool; its activity could be explained by the presence of γ-terpinene (23.1%) which is known for its antimicrobial potential through a strong bacterial membrane lipid content leakage ability [30]. The greater activity of the Burkinabe chemotype of *L. multiflora* can also be attributed to thymol acetate (3.6%) and carvacrol (4.1%), two phenolic derivatives with proven antibacterial properties which were present in the Burkinabe chemotype.

Regarding the screening on *Pseudomonas aeruginosa*, only two EOs, namely *C. giganteus* and *E. camaldulensis* from Benin, were active on the wild strain PAO1 (with MIC $<$ 1% v/v). It is necessary to remember that *P. aeruginosa* is known to be resistant to most of first-line antibiotics mainly because of its low outer membrane permeability and its active antibiotic efflux pumps . The *C. giganteus* EO activity was also reported by Jirovetz et al. in 2007 who found a MIC of 0.006% v/v on *P. aeruginosa* strain G28 by agar dilution method; they attributed this activity to the presence of *p*-menthadienols also found in the chemotypes studied here [27]. The activity of *E. camaldulensis* EO on strain PAO1 could be attributed at least in part to the presence of terpinen-4-ol (4.6%), found to be effective on *P. aeruginosa* [32]. Apart from this last EO, the EOs tested were more active on the deleted strain than on the wild strain of *P. aeruginosa*. This suggests that the active compounds of these EOs are sensitive to the efflux mechanisms developed by *P. aeruginosa* contrary to the observations made in the case of the resistance developed by the MRSA strain of *S. aureus*.

Although we have demonstrated an interesting antibacterial potential for most of the EOs in the study, it should be noted that they present an activity from 40 to more than 10^4 times lower than that of the reference antibiotics tested on the same strains (Table 5). Nevertheless, EOs are mixtures of compounds, so some of them may possess a higher activity than the mixture.

We also have to point out that some EOs whose antimicrobial activity is commonly reported in the literature showed in our study a very low antibacterial potential. This is the case of *M. piperita*'s EO whose sample did not show activity (MIC $>$ 1%), however interesting antimicrobial activities were reported in literature on *S. aureus* and *P. aeruginosa* [22,33]. This discrepancy with the literature data is due to the absence of menthol in the chemotype studied here, unlike the usually recognized variety which can contain up to 65% menthol. Indeed, according to Iscan et al., menthol associated with menthone represents the main pharmacophore [33]. The same observation was made for *C. ambrosioides* EO. While our study did not show any inhibitory activity on *S. aureus*, Alitonou et al. in 2012 [34] found an activity (MIC = 0.17% w/v) on MSSA ATCC 25923 by broth microdilution method. However, it should be noted that in the chemotype used by Alitonou et al., the major component is α-terpinene (48.8%–63.7%) which is not present in the chemotype we studied, whose major compound is *p*-cymene (47.1 %). This shows that literature data cannot be applied to all samples of EOs from the same species grown in different conditions/climates and the importance of the studied chemotype.

It is also appropriate to discuss the impact of polysorbate 80, the surfactant used to disperse EOs in the nutrient broths. No inhibition of the growth of reference or clinical strains was shown by polysorbate 80 at its final concentrations in the culture broths. However, some authors have shown that polysorbate 80 even added at concentrations as low as 0.1% to laboratory culture media could increase the planktonic growth rate of bacteria such as *S. aureus*; an effect that could vary or be reversed depending on the bacteria [35]. The same authors also suggested that polysorbate 80 may also reduce the efficacy of hydrophobic antimicrobials during *in vitro* tests. However, without neglecting the existence of a potential bias, it should be recalled that for therapeutic cutaneous application of EOs, delivery vehicles are often emulsion-type matrices containing surfactant such as polysorbate 80. It therefore appears useful to carry out *in vitro* tests under such conditions,

Table 6
Antimicrobial screening of the most effective EOs on clinical isolates obtained from skin infections

EOs	*C. citratus*		*C. nardus*		*C. giganteus*	*O. gratissimum*	*O. basilicum*	*L. multiflora*	*E. camaldulensis*
	BF	BN	BF	BN	BN	BF	BF	BF	BN
BACTERIA									
S. aureus	0.13	0.13	0.25–0.5	0.25	0.13	0.13–0.25	0.25	0.25	0.13–0.25
S. epidermidis	0.35	0.35	0.5–1	0.25–0.5	0.13–0.25	0.35	0.5	0.5	0.25–0.5
A. baumannii	0.25–0.5	0.5	0.25	0.25–0.5	0.03–0.06	0.13	0.13–0.25	0.25	0.13–0.25
P. aeruginosa	>1	>1	>1	>1	0.5–1	>1	>1	>1	0.5
S. pyogenes	0.06-0.13	0.13	0.13	0.06	0.13	0.06-0.13	0.06	0.13	0.25
YEASTS									
YS-04	-	-	0.06	0.06	0.03	0.02	-	-	-
YS-05	0.25	1	1	>1	0.13	0.13	-	-	-
YS-08	0.02	0.06	0.13	0.25	0.02	0.13	-	-	-
YS-61	-	0.02	0.13	0.13	0.06	0.06	-	-	-
YS-68	-		0.50	0.50	0.03	0.25	-	-	-
YS-98	-		0.13	0.13	0.06	0.13	-	-	-
YS-133		0.13	0.25	0.13	0.06	0.06	-	-	-
YS-151	-	0.13	0.25	0.13	0.06	0.13	-	-	-
YS-07	0.02	0.13	0.13	0.13	0.02	0.13	-	-	-
YS-26	0.13	0.13	0.25	0.25	0.03	0.25	-	-	-
YS-09	0.25	0.25	0.5	0.5	0.06	0.25	-	-	-
YS-78	0.03	0.13	0.06	0.06	0.03	0.06	-	-	-
YS-93	-	0.03	-	0.13	0.06	0.06	-	-	-
YS-119	-	-	0.008	0.13	<0.0075		-	-	-

The MIC values are expressed in % vol/vol (n = 3); BN, Benin; BF, Burkina Faso

especially since the effect of the other solubilizing or dispersing agents is less known. Indeed, many authors recommend the use of polysorbate 80 as an emulsifier during *in vitro* tests, finding the results very consistent [20].

3.2. Antimicrobial Activities of the Most Effective EOs on Clinical Isolates Strains of Bacteria and Yeasts Involved in Common Skin Infections

The nine most active EOs from our screening on reference strains were tested on bacteria isolated from clinical lesions. MIC values observed in the first screening were largely confirmed on staphylococci as shown in Table 6. The results were even more interesting than expected on the multidrug-resistant strain of *A. baumannii*. Indeed, at least one sample of each EO proved to be active with MIC < 0.35% v/v. Hammer et al [25] had also found by agar dilution method on *A. baumannii*, a high activity of *C. citratus* EO (MIC = 0.03% v/v) on strain ATCC 15308 and of *C. nardus* EO (MIC = 0.25%) on strain NCTC 7844. In our study, *C. giganteus* EO was even more active with a MIC value of 0.04% v/v on this strain.

The low potential of the tested EOs on *P. aeruginosa* already observed on the wild reference strain PAO1 is here confirmed. However, the EOs of *C. giganteus* and *E. camaldulensis* retained their activity on *P. aeruginosa* clinical isolates.

The tested EOs showed low MIC values on the strain of *S. pyogenes*. Several studies had shown EOs activity on this bacterium. Thus Inouye et al in 2001 [23] on the one hand and Derbré et al in 2013 [36] on the other hand had proven the activity of *C. citratus* EO by broth dilution method respectively on *S. pyogenes* strains ATCC 12344 (0.04% w/v) and CIP 104226 (0.93% v/v). Rasooli et al. in 2008 [37] showed the activity of *E. camaldulensis* EO on an unspecified strain of *S. pyogenes*.

The tested EOs showed a remarkable potential on the yeast strains. The anti-candida activity of EOs was already well known in the literature. Thus Hammer et al had demonstrated by agar dilution method the activity of EOs from *C. citratus* and *C. nardus* on the *Candida albicans* strain ATCC 10231 with MICs of 0.06% v/v and 0.12% v/v respectively [25].

In our study, EO from *C. giganteus* was most active on yeasts with MIC < 0.0075% v/v on the isolate YS-119. Jirovetz et al in 2007 [27] also obtained a MIC value of 0.006% v/v on the reference strain ATCC 10231 of *C. albicans*.

The inhibitory activity of EOs of *O. gratissimum* and *O. basilicum* was shown by Hzounda et al in 2014 by broth microdilution method on *C. albicans* strains ATCC 12C and ATCC L26 respectively [38]. Oladiméji et al in 2004 demonstrated the activity of EO of *L. multiflora* on a clinical isolate of *C. albicans* [39].

Several cytotoxicity studies on cutaneous cell lines of the most active EOs identified in this study reported that cytotoxic concentrations are significantly higher than the MICs values determined here on bacteria and yeasts. The same observation was reported for the isolated terpenic compounds. For example, in a study carried out on West African *C. citratus* and *C. nardus* EOs from Togo, the *in vitro* cytotoxicity bioassays on the human epidermic cell line HaCaT showed respective IC50 values of 15% v/v and 45% v/v. Likewise, pure commercial neral, geranial and citronellal standards showed IC50 values of 10%, 25% and 30% v/v respectively [40].

4. Conclusion

Starting from a group of plants either used empirically for the treatment of skin diseases in West Africa or/and plants with recognized antimicrobial properties, we tried during this study, to determine the most active samples from Benin and Burkina Faso on bacteria and yeasts involved in skin infections. The EOs extracted from nine samples of these aromatic plants were found particularly active. These were EOs extracted from plant organ samples of *C. citratus* and *C. nardus* collected in both Benin and Burkina Faso, those of *C. giganteus* and *E. camaldulensis* collected in Benin and those of *O. gratissimum, O. basilicum*, and *L. multiflora* collected in Burkina Faso. Even if the highlighted antimicrobial activities were lower than antibiotics ones, EOs active concentrations can be easily obtained locally. In the same way, the wide variety of active components in these EOs represent a very valuable asset in the search for therapeutic alternatives to deal with the increasing multidrug resistance of germs involved in common skin infections.

Authors Contribution

HS conducted all the experiments with the contribution of HT for experiments on reference strains and for phytochemical analysis. LC contributed for experiments on reference strains. FVB supervised the microbiology work in Belgium and FT supervised the microbiology work in Benin. JQL and FG supervised and coordinated all the experiments. JQL, FG and BE were the promoters of the study. HS, supervised by JQL, wrote the draft article and all authors contributed to the editing of the manuscript.

Declaration of Competing Interest

The authors state no conflict of interest.

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