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ASSESSMENT OF FOUR ESSENTIAL OILS AGAINST BACTERIAL SPECIES ISOLATED FROM PATIENTS WITH ORAL INFECTION

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ABSTRACT

Oral micro-organisms play a significant role in mouth disease. The aim of this work was to evaluate the antimicrobial potency of four essential oils against seven bacterial species isolated from patients sickly of oral infections. Bacterial species were isolated and identified from patient sample during a prospective study at the Yaounde University Teaching Hospital Centre. The essential oils were analysed simultaneously by Gas Chromatography and Gas Chromatography / Mass Spectrophotometry. Agar disk diffusion and microdilution methods were used to assay the antibacterial activity. The highest inhibition zone was recorded against *Staphylococcus aureus* (43.5 ± 3.17) for *Pentadiplandra brazzeana*. The highest minimum inhibitory concentration (0.078mg/mL) values were detected for *Klebsiella pneumonia* and minimal bactericidal concentration (0.078 mg/mL) values were detected for *Eugenia caryophylla*. The active essential oils contained mainly hydrocarnated and oxygenated monoterpens. Active essential oils represent a potential source of antibacterial substances in mouthwash and dentifrice.

KEYWORDS

Oral infection, Essential Oils and Antimicrobial activity.

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INTRODUCTON

Oral diseases continuous to be a major health problem worldwide. The link between oral diseases and the activities of microbial species that form part of the microbiota of the oral cavity is well established. Over 750 species of bacteria inhabit the oral cavity and a number of these are involved in oral diseases[1]. Interactions between these microorganism cause oral infections, the most common of which are tooth decay and periodontal diseases[2]. Several factors are at the origin of these

oral diseases, this is the case with the accumulation of dental plaque, dryness of the mouth. Above all, poor oral hygiene, which favor the development of cariogenic bacteria and other pathogenic bacteria in the mouth[3].

Several agents for the treatment of oral diseases are commercially available for example the antibiotics commonly used to treat oral infections such as: Penicillins and Cephalosporins, Erythromycin, Tetracycline and derivatives have been documented[4]. These chemicals can alter oral microbiota and have undesirable side effects such as vomiting, diarrhea and tooth staining[5]. Other antibacterial agents used in the prevention and treatment of oral diseases including Cetylpyridinium chloride, Chlorhexidine, Amine fluoride or products containing such agents are reported to exhibit toxicity, cause staining of teeth or in the case of ethanol (commonly found in mouth washes) have been linked to oral cancer[6]. Given the incidence of oral disease, increased resistance by bacteria to antibiotics, adverse effects of some antibacterial agents currently used in dentistry and financial considerations in developing countries, there is a need for alternative prevention and treatment options that are safe, effective and economical. The use of essential oils of aromatic and medicinal plants of the Cameroonian Pharmacopoeia, like *Cymbopogon citratus* DC. Staf (Poaceae), *Eugenia caryophylla* (Myrtaceae), *Mentha sp cf piperita* (Lamiaceae) and *Pentadiplandra brazzeana* Baill (Capparidaceae) could be an approach of choice.

Cymbopogon citratus (lemon grass) is a plant with thin and linear leaves coloured green releasing a fresh and relaxing smell. It is a traditional cooking ingredient in Cameroon and its leaf extracts are widely used as relaxing herbal tea which helps to fight against stress, relieve stomach aches. In Central Africa, *Cymbopogon citratus* is mostly cultivated around houses because the flavour of its leaves is toxic for mosquitoes[7]. Concerning *P. brazzeana*, it shrub or climber has 3 to 20 m of average size and the leaves are largely elliptic, oblong, oval to lanceolate, acute or rounded at the base, obtuse and long acuminate, measuring 5 to 15 cm long and 1 to 7 cm wide. It is a very common and quite common plant (secondary and primary forests) found in Western and Central Africa, the berries of which are eaten and used as a sweetener of beverages. Root, seed and leaf extracts of *P. brazzeana* are known to contain glucosinolates[8,9] and the essential oil obtained from its roots is mainly constituted of benzyl isothiocyanate, phenyl acetonitrile and benzyl cyanide[9]. Eugenol, major component of *Eugenia caryiophylla* has been used topically in dental practice to relieve pain arising from a variety of sources, including pulpits and dentinal hypersensitivity. Interestingly, eugenol exhibits irritant action in addition to its analgesic effect as found in certain studies[10]. These plant derived medicines used in traditional medicinal systems have been recorded in Cameroonian pharmacopoeia as agents used to treat infections, and a number of these have been recently investigated for their efficacy against microbial pathogens[11].

Plant extracts or phytochemicals that inhibit the growth of oral pathogens, reduce the development of biofilms and dental plaque, influence the adhesion of bacteria to surfaces and reduce the symptoms of oral diseases can serve as alternatives in prevention and treatment of dental caries[12]. In this study, we evaluate the antibacterial potency of *Eugenia caryophilla*, *Cimbopogom citratus*, *Mentha sp cf piperita* and *Pentadiplandra brazzeana* essential oils against seven bacterial species isolated from patient with oral infection.

MATERIAL AND METHODS

Sample collection

This study is a prospective study that took place in laboratory of Bacteriology, Training University Hospital Centre (Yaounde-Cameroon) from April to December 2017, after obtaining authorization from Ethics Committee (Nº 2018/02/1070/L/CNERSH/SP). Samples were collected from 31 patients, 18 females and 13 males between the age group of 18 to 60 years. Samples were collected from a private dental clinic “La rose” (Yaounde –Cameroon) after a written informed consent was taken from the patient. Samples collected in sterile wide-mouthed screw capped tubes containing 5 ml of sterile Brain Heart Infusion Broth (TM MEDIA, India). Immediately the

sampled carried to the laboratory and processed within 2 hours of collection.

Patient sample characteristics

Patient who are include in the study must agree to participate and age between 18 to 60 years. These patient must be in good general health, with identified oral infection who came in dentist consultation. All patient with antimicrobial therapy within past 1 month, or who had used dentifrice contening fluor or Chlorexidin 72 hours before was exclude.

Isolation of organisms

The clinical samples were homogenized by a vortex mixer and it was streaked on sterile Columbia agar plate (Scharlau, Spain) with 5% blood, Chapman (Liofilchem, Italia) agar plate and Eosin Methylen Blue (EMB) agar plate (TM MEDIA, India). The plates were incubated for 24 hours at 37 °C.

After incubation, the macroscopic appearance of the different colonies were noted (size, shape, relief, contour, surface, opacity, color and consistency) and individual transplantation of the majority colony was made on the same media and incubated under the same conditions. The purity of the strains after a succession of subcultures was confirmed by the uniform appearance of the microbial colonies on the culture medium and by microscopic tests (fresh state and Gram stain). All the purified isolates were maintained on sterile Muller Hinton agar slants.

Identification of Bacterial species

Microbial isolates were identified according to Bergey's Manual of Systematic Bacteriology[13] and the book of Technical Microbiology[14], beginning with macroscopic and microscopic tests (fresh state, Gram stain), followed by biochemical test.

Extraction and chemical analyses of essential oils

For essential oil extraction, *Cymbopogon cytratus* and *Pentadiplandra brazzeana* has been harvest while *Eugenia caryophylla* and *Mentha sp cf Piperita* were purchased and identified at the National Herbarium of Cameroon (Table No.1). The essential oils of each plant materiel were extracted by hydrodistillation using a Clevenger-type apparatus for 5 h, dried over anhydrous sodium sulphate and then 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 and expressed as a percentage[15].

Eos were analyzed by Gas Chromatography–Flame Ionization Detector (GC-FID) and Gas Chromatography coupled with Mass Spectrometry (GC/SM) as described by Agnaniet *et al*[16].

GC analysis was performed on a Varian gas chromatograph, model CP-3380, with flame ionization detector containing two silica capillary columns: HP5 J and 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. × 0.25µm film); N2 was the carrier gas at 0.8mL/min; injection type 0.1µL 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.

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 x 0.25 mm, film thickness 0.25µm). Helium was the carrier gas, at a flow rate of 0.6mL/min. Injector and MS transfer line temperatures were 220°C and 250°C, respectively. The oven programme 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 70eV, in the m/z range 35-300; electron multiplier 1460 eV; scan rate, 2.96 scan/s.

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)[17,18].

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[17,18].

Sensitivity of the microbial species to essential oils

Agar disk diffusion test

The bacterial inoculum of each clinical isolate was prepared from fresh colonies grown on Muller Hinton (Scharlau, Spain) agar slants. Each bacterial strain was introduced into 5mL of Muller Hinton broth (L: S-BIOTECH® CA9212 USA) in order to obtain a concentration of 1.5 x 10^8 CFU/mL (0.5 Macfarland turbidity). In order to support obtaining a tablecloth of semi colony–confluent, the bacterial inoculum of known title was shown on Mueller Hinton agar plates (Scharlau, Spain). Sterile filter paper disks (Schleicher, Germany, 6 mm in diameter) was impregnated with 10 µL of EOs diluted in 10% DMSO (Merck), working concentration (50 mg/mL). Gentamicin® (2mg/mL) were use as the antibacterial reference control. The plates were incubated at 37°C for 24 hours according to conditions suitable for the growth of microorganism. The absence of bacterial growth around each disc expressed an antimicrobial activity illustrated by a translucent halo of the same color than the sterile medium. This diameter expressed in millimeter (mm) was measured using a slide caliper or a scale[19]. All tests were performed in triplicate.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of essential oils

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for clinical isolates. MIC and MBC were determined using the microdilution reference method recommended by the Clinical Laboratory Standards Institute (CLSI), with modifications. 100 µL of sterile Muller Hinton broth (L: S-BIOTECH® CA9212 USA) was dispensed into the 96 well microtiter. A serial doubling dilution of different EOs (1.25-0.039 mg/mL) and Gentamicin® (0.5-0.062 mg/mL) was realised. EOs was supplemented with DMSO (Merck) at a 10% concentration in order to enhance sample solubility. The cultures with visible turbidity adjusted to approximately 1.5 x10^8 CFU/mL (0.5 Macfarl and turbidity) in Muller Hinton broth were supplemented with different concentrations of the compounds tested and incubated at 37ºC for 24 hours. Positive and negative control wells were included and all tests were done in triplicate.

The MBC was assessed by subculture. 50 µL of the content of wells (unrevealed) corresponding to concentrations ≥ MIC were transferred unto 150 µL of fresh Muller Hinton broth. The plates were incubated at 37ºC for 48 hours. 40 µL of alamar blue (0.5%) was used to reveal bacterial growth in each well. The MBC was regarded as the lowest concentration of each antibacterial substance that did not allow any noticeable color change from blue to yellow[20]. All tests were performed in triplicate.

Statistical analysis

Data were combined and analysed by analysis of variance (ANOVA). The ANOVA was performed with SPSS software (version 23.). The significant differences ($p<0.05$) were estimated by Tukey and values were expressed as mean ± SD.

RESULTS

Phenotypic identification of strains isolated from oral samples

Frequencies of individuals sampled

Oral samples were collected from 31 patients including 18 females and 13 males. The type of infection was noted for each patient and the value of the total number of patients affected was expressed as a percentage (Table No.2). The result showed that tooth decay is the most represented disease with a frequency of 48.3 %. The second oral pathology was pulpitis with a total frequency of 29.0 %. The cases of periodontal abscess, apical granuloma and periodontal pocket were also noted with a frequency equal to 6.5%. Pericoronitis is the least observed infection with only 3.2% of the patients sampled.

Identified genus or microbial species of each infection

Depending on each type of infection, the phenotypes of the isolated microorganisms were investigated via microscopic, macroscopic observations and biochemical tests (Table No.3). This table represents for each infection, the genus and bacterial species obtained and classified according to their Gram type and their shape. It appears that in every oral pathology Gram + and Gram - bacteria are found, with the exception of gingival abscess, where only Gram + bacteria are

observed. Cocci and bacilli are the representative bacterial forms of all infections, except for cases of periodontal abscess where only cocci are observed. With respect to microbial diversity, tooth decay and pulpitis are diseases with greater etiological diversity. Furthermore, we notes that *Staphylococcus* species was present in all types of infections. *Streptococcus* species which is frequently cited as a causative agent in oral diseases, was found in tooth decay, pulpitis and periodontal pockets. *Bacillus*, *Klebsiella* and *Enterobacter* genus, which are also fairly mentioned, have been identified in cases of tooth decay and dental pulpitis. *Citrobacter*, *Listeria* and *Proteus* genus almost not mentioned in oral pathologies are identified.

Distribution of isolates identified by microbial type

After microbial isolation from 31 patients with oral diseases, 13 bacterial germs were identified. These were divided according to their Gram type, as well as their shape and species. It appears that on all the isolates, the bacteria represent 80 % (Figure 1A). Concerning identified bacterial isolates, 34% are Gram + cocci, 30% Gram - bacilli and 16% Gram + bacilli (Figure No.1B).

Frequencies of identified species

The frequency of each microbial species was determined for all the isolates obtained (Table No. 4). It appears that the majority species is *Staphylococcus aureus* and *Bacillus cereus* with frequency of 8.20% each. The species *Streptococcus viridans* and *Enterobacter cloacae* are also well represented in our microbial isolates with a frequency of 6.56% each. Species such as *Staphylococcus hominis*, *Staphylococcus simulans, Klebsiella pneumonia* and *Proteus mirabilis* have a frequency of 4.92% each. Other species such as *Bacillus subtilis* and *Klebsiella oxytoca* had a frequency of 3.28%. The least represented species with a frequency of 1.64% are *Enterococcus* spp., *Streptococcus* spp., *Listeria murrayi*, *Corynebacterium* spp., *Corynebacterium striatum*, *Actinobacillus* spp., *Actinomyces* spp., *Aeromonas* spp. and *Proteus rettgerii*.

Analysis of essential oils

Extraction yields of essential oils

The extraction yields of EOs of *Eugenia caryophylla* has showed the highest yield 1.7% compared to those of *Cymbopogon citratus*, *Pentadiplandra brazzeana* and *Mentha sp cp piperita* which were 0.3%, 0.05% and 0.04% respectively.

Chemical composition of essentials oils

The results of the chromatographic analysis are summarized in the Tables N^{O} 6**.** The analysis of *Cymbopogon citatus* show that the major components are geranial (33.8 %) and neral (24 %). The analysis of *Eugenia caryophylla* show that the major components are eugenol (70 %) and eugenol acetate (16.2%). The analysis of *Mentha sp cf piperita* show that the major components are piperitone (50.7 %), β-phallandren (21 %) and methyl acetate (19.3%). The analysis of *Pentadiplandra brazzeana* show that it is composed mainly of benzyl isothiocyanate (86.8 %).

Sensitivity test

Agar disk diffusion test

Seven (07) bacterial isolated whish are most representative in oral infection of patient including *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter cloacae*, *Klebsiella pneumoniae, Klebsiella oxytoca*, *Staphyloccoccus aureus* and *Streptococcus* spp were used to evaluate the antibacterial effect of EOs. The result from the diameter inhibition zone observed were summarized in table No.6. This table show that *P. brazzeana* EO is the most active on all isolates tested. *Staphylococcus aureus* species was most sensitive to *P. brazzeana* EO with inhibition diameter of 43.5 ± 3.17 mm. *Mentha cf sp piperata* EO is the least active because it had no effect on several isolates including *Bacillus cereus*, *Bacillus subtilis*, *Klebsiella oxytoca* and *Staphylococcus aureus*.

Determination of MIC and MBC

The minimum inhibitory concentration of the EOs vary according the type of germ and range from 0.07mg/mL to 1.25mg/mL. The EOs of *Pentadiplandra brazzeana* and *Eugenia caryophylla* were most effective against *Klepsiella pneumonia* (0.07mg/mL) and *Basillus cereus* (0.31 mg/mL). Contrarily, the EOs of *Cymbopogon citratus* and

Mentha sp cf piperita were the least efficient (Table No.7).

DISCUSSION

The results of phenotypic identification of microbial isolates from this study showed the abundance of facultative aero-anaerobic Gram-positive cocci with a predominance of the genus *Staphylococcus* and *Streptococcus*. These results are in agreement with those of Kammogne[21] who obtained a frequency of 55 % of *staphylococci*. In the group of Gram positive cocci belonging to the genus *Staphylococcus*, we noted the presence of the species *Staphylococcus aureus* with a frequency of 8.2 %. Schupbach[22] reported the isolation frequencies of *Staphylococcus aureus* in primary and advanced carious lesions (4.4 % and 15.5 % respectively). Smith and Storoe found significant presence of *Staphylococcus aureus* in dental infections and odontogenic infections resulting in swelling of the face or neck[23,24]. For the coagulase-negative s*taphylococci* identified in this study, the most common species was *Staphylococcus epidermidis* with a rate of 6.56 %. This result is in contradiction with that of Jussara[25], who was 42.17% *Staphylococcus epidermidis* in 88 patients with periodontal infections. The two majority species obtained from our clinical samples were *Bacillus cereus* (8.2%) and *Bacillus subtilis* (3.28%). They are sporulated positive Gram bacilli, belonging to the *Bacillaceae* family. The spore may be terminally, subterminally or centrally possessing a heat resistant. They are emerging opportunistic pathogens, usually associated with food poisoning or local infections of the eye and periodontium. Their involvement in the infectious process of the oral cavity is not fully described. Nevertheless, it is possible that the species Bacillus cereus contributes to the formation of the oral biofilm by adhering to surfaces to form dental plaque[26]. They also cause periodontitis and gingivitis. The oral cavity can serve as a potential reservoir for *enterobacteria*, which under certain conditions are predisposing factors and aggravates oral pathologies[27]. It has been reported that the prevalence of enteric bacilli in the oral cavity is related to oral health[28]. In our study, the proportion of *Enterobacteriaceae* was high at 30 %. The predominantly isolated and identified species were: *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Proteus* with respective rates of 6.56 %, 4.92 %, 3.28 % and 4.92 %. According to Nelson[29], pathogens such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* can be found in the subgingival sites of patients with periodontitis. Santos[30] reported that *Enterobacter cloacae* and *Klebsiella pneumoniae* were most common in individuals with dental involvement. The results of the identification of sprouts responsible for dental abscesses in the Ghada study[31], revealed the presence of microbial species such as *Proteus mirabilis*, *Listeria grayi*, *Staphylococcus aureus* and *Clostridium perfringens*.

The *Pentadiplandra brazzeana* oil contained predominantly 86.8 % benzyl isothiocyanate. These results are similar to the result of Nyegue[32] and Kamela[33] who obtained the same compound with respective rates of 78 % and 91.27 %. The formation of benzyl isothiocyanate depends on the pH of the medium. If it is neutral, a benzyl isothiocyanate is formed, very reactive, volatile, with a strong odor and bitter taste, if it is acidic, nitrile is formed if it is basic, thiocyanate is formed[34]. *Eugenia caryophylla* EO consisted mainly of eugenol (70.0 %), eugenol acetate (16.2 %) and β-caryophyllene (5.2 %). This composition is similar to that obtained by Nyegue[37] who showed that the EOs of *Eugenia caryophylla* buds from different harvesting locations would maintain the same profile and composition of *Eugenia caryophylla* would be between 70-90 % for eugenol, 17 % for eugenol acetate and 12-15% for β-caryophyllene. The chemical composition of the essential oil of *Eugenia caryophylla* varies very slidely despite the different site of harvest. Chromatographic analysis of the essential oil of *Cymbopogon citratus* revealed geranial (33.8 %), neral (24 %), nerol (8.5 %), geraniol (7.3 %) and myrcene (6.9 %). These results are similar to those generally found in the literature. The work of Yayi-Ladekan[35] done on the essential oil of *C. citratus* showed the main constituents to be: geranial, neral, β-pinene, cis-geraniol, cis-verbenol and geranyl acetate. Ndoye[36] showed that the *C. citratus* phenotypes of Lomié and Longtsimbi were similar to those of Batourie in citral levels. This shows us

that the type of compound presenting high quantity and its content is very varies slidely variable depending on the location of harvest of plant material. The EO of *Mentha sp cf piperita* consisted mainly of pipetorine (70%), β-phallandrene (15.6 %), menthyl acetate (19.29 %) and myrcene (1.43 %). Its major compound obtained being piperitone is same as that obtained by Nyegue[37], confirming the identification of *Mentha sp cf piperita* as a new mint chemotype[37]. And differs from the result of Benayad[38] and Derwick[39] who respectively obtained menthol and menthanol as major compounds. This variability of major compound could be due to the geographical origin of the plant[40].

According to the results of the antimicrobial tests of essential oils, we noted that the essential oil of *Pentadiplandra brazzeana* was the most active. Its diameters of inhibition obtained were much greater on *Staphylococcus aureus* (43.5 ± 3.17mm). These results are twice as high as those of Kamela[33] who reported diameters of 22 mm at concentration of 30 mg / ml of EO on the same germ. This divergence of results is due to the different concentrations used for each study. Bacterial strains such as *Enterobacterium cloacae*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were also susceptible to *P. brazzeana* essential oil with inhibition diameters of 39.5 mm, 42 mm and 43.5 mm respectively. The antimicrobial power of this plant comes from its bioactive components namely benzyl isothiocyanate and p-methoxybenzaldehyde.

The MIC of EOs varies from 0.07 to 1.25 mg/mL. The most active EO was that of *Pentadiplandra brazzeana* on majority of bacterial strain tested with bactericidal effect (MIC/MIB=1), followed by *Eugenia caryophylla.* The most sensitive microorganisms were *Streptococus spp* and *Klepsiala pneumonia*. The most resistant was *Bacillus cereus*. The observed activity is probably due to the synergistic interaction between the compounds within the EOs endowed with antibacterial activity just to cite hydrocarbon monoterpenes, oxygenated monoterpenes and sesquiterpene. In general, the Gram negative bacteria proved to be more resistant to the tested EOs products than the Gram positive bacteria. This marked sensitivity demonstrated by Gram-positive bacteria to the EOs compound have been observed by several authors[5].

Table No.1: Plant used for essential oil extraction

S.No	Name of the plant	Collection date /Location harvest	Part used	Identification number
1	*Cymbopogon citratus* DC. Stapf (Poaceae)	April 2017/Yaounde-Cameroon, Center Region	Leaves	48536/SFR/Cam
2	*Eugenia caryophylla* (Myrtaceae)	May 2017/Bamendjou-Cameroon, West Region	Floral buttons	506167/SRF/Cam
3	*Menta cf sp piperita* (Lamiaceae)	Jully 2017/Yaounde-Camenroon, Center Region	Leaves	25t45/SRF/Cam
4	*Pentadiplandra brazzeana* Baill. (Capparidaceae)	May 2017/Dschang-Cameroon, West Region	Roots	42918/SRF/Cam

Table No.2: Frequency of individuals sampled according to the type of oral infection

		Oral infections						
S.No	**Patients**	**Tooth decay**	**Pulpitis**	**Periodontal abscess**	**Apical granuloma**	**Pericoronitis**	**Periodontal pocket**	**Total**
1	Number of patients affected	15	9	2	2	1	2	31
2	Frequencies of Patients (%)	48.3	29.0	6.5	6.5	3.2	6.5	100

Table No.3: Bacterial species isolated from different type of oral infection

S.No	Types of infections	Gram type	Shapes	Names of the genus or species of the microorganism
1	Tooth decay	Gram +	Cocci	*Staphylococcus aureus, Staphylococcus* spp*, Staphylococcus epidermitis*, *Streptococcus* spp
			Bacilli	*Listeria murrayi, Bacillus* spp*, Bacillus subtilis*
		Gram -	Bacilli	*Klebsiella pneumoniae, Enterobacter cloacae, Klebsiella oxytoca,*
2	Pulpites	Gram +	Cocci	*Staphylococcus aureus, Staphylococcus* spp. *Staphylococcus epidermitis, Streptococcus* spp
			Bacilli	*Corynebacterium diphteria, , Corynebacterimum striatum, Bacillus coagulans,*
		Gram -	Bacilli	*Klebsiella oxytoca, Actinobacillus* spp. *Proteus mirabilis, Klebsiellap pneumoniae*, *Proteus rettgeri, Citrobacter freundii. Actinobacillus sius, Aeromonas* spp*, Actinomyces* spp.
3	Periodontal abscess	Gram +	Cocci	*Staphylococcus* spp, *Staphylococcus simulans, Streptococcus* spp
4	Apical granuloma	Gram +	Cocci	*Staphylococcus epidermidis, Staphylococcus* spp
		Gram -	Bacilli	*Proteus mirabilis*
5	Pericoronitis	Gram +	Cocci	*Staphylococcus* spp,
		Gram -	Bacilli	*Pasteurella* spp
6	Periodontal pocket	Gram +	Cocci	*Staphylococcus* spp,
		Gram -	Bacilli	*Citrobacter freundii, Enterobacter amnigenus 1, Klebsiella pneumonia*

Table No.4: Frequencies of identified species in all isolates obtained

S.No	Type of microorganisms		species identified	Frequencies of isolats (%)
1	Gram + (cocci)	positive catalase	*Staphylococcus aureus*	8.20
			Staphylococcus epidermidis	6.56
			Staphylococcus hominis	4.92
			Staphylococcus simulans	4.92
		négative catalase	*Streptococcus viridans*	6.56
			Enterococcus spp	1.64
			Streptococcus spp.	1.64
2	Gram + (bacillus)	Sporulated	*Bacillus cereus*	8.20
			Bacillus subtilis	3.28
		Not sporulated	*Listeria murrayi*	1.64
			Corynebacterium spp	1.64
			Corynebacterium striatum	1.64
3	Gram – (bacillus)		*Actinobacillus* spp	1.64
			Actinomyces spp	1.64
			Aeromonas spp	1.64
			Enterobacter cloacae	6.56
			Enterobacter amnigenus 1	1.64
			Klebsiella oxytoca	3.28
			Klebsiella pneumoniae	4.92
			Proteus mirabilis	4.92
			Proteus rettgerii	1.64

Table No.5: Chemical profile of essentials oils

S.No	Compound	LRI	Percentage (%)			
			P. b	***M. p***	***E. c***	***C. c***
1	α-Pinene	940	-	0.6	0.4	2.4
2	Camphene	943	-	-	-	0.3
3	Sabinene	957	-	-	-	1.8
4	Benzaldehyde	963	0.2	-	-	-
5	β-Pinene	985	-	0.7	0.7	1.0
6	Myrcene	995	-	1.4	-	6.9
7	(Z)- β –Ocimene	1038	-	-	0.6	-
8	β-Phellandrene	1043	-	21.0	-	-
9	*Trans*-sabine hydrate	1047	-	-	-	1.3
10	(E)-β-Ocimene	1049	-	0.2	-	0.3
11	*Cis*- sabinene hydrate	1062	-	0.1	-	-
12	Terpinolene	1090	-	-	1.7	1.0
13	Linallol	1103	-	-	-	1.5
14	2,3- Octa-3,4-dienal	1115	-	-	-	0.2
15	Benzyl cyanide	1143	2.7	-	-	-
16	Camphor	1144	-	-	-	0.8
17	Citronellal	1151	-	-	-	0.3
18	Cis-Pinene hydrate	1161	-	0.3	-	-
19	Berneol	1164	-	-	-	1.2
20	Menthol	1170	-	0.3	-	-
21	Terpinen-4-ol	1183	-	-	-	2.6
22	*p*-Menth-1,5-dien-8-ol	1186	-	0.4	-	-
23	Nerol	1248	-	-	-	8.5
24	Linalool acetate	1252	-	-	0.3	-
25	Menthyl acetate	1257	-	19.3	-	-
26	Neral	1262	-	-	-	24.0
27	Geraniol	1279	-	-	-	7.3
28	Piperitone	1277	-	50.7	-	-
29	Trans sabinyl acetate	1289	-	-	0.7	-
30	Geranial	1297	-	-	-	33.8
31	Geranyl acetate	1301	-	-	-	0.3
32	α-Terpineol acetate	1344	-	0.1	-	-
33	Dihydrocarveol acetate neosi	1355	-	0.1	-	-
34	*p*-Methoxybenzaldehyde	1370	9.1	-	-	-
35	(E)-Caryophyllene	1377	-	-	-	0.2
36	Undecanol <n->	1388	-	0.3	-	-
37	β-Cubunene	1391	-	0.1	-	-
38	Benzyl isothiocyanate	1403	86.8	-	-	-
39	α-Humulene	1424	-	0.7	-	-
40	Eugenol	1440	-	-	70.0	-
41	Carvyl propanoate trans	1447	-	0.3	-	-
42	Muurolene	1456	-	0.1	-	-
43	γ-Amorphene	1465	-	0.2	-	-

44	*β* – Caryophyllene	1469	-	-	5.2	-
45	(E)-*β*-Ionone	1484	-	-	-	0.5
46	Germacrene	1486	-	0.9	0.2	0.4
47	*β*- Caryophile	1497	-	0.1	-	-
48	<*γ*>- Cadinene	1510	-	-	0.7	-
49	*δ*-Cadinene	1521	-	0.1	-	0.2
50	Sesquisabinene hydrate<trans>	1536	-	0.1	-	-
51	Eugenol acetate	1537	-	-	16.2	-
52	Sesquithujene	1595	-	0.1	-	0.3
53	Naphtalene <2- acetyl->	1597	-	-	0.6	-
54	*p*-Methoxybenzyl isothiocyanate	1603	1.0	-	-	-
55	Cinamaldehyde<hydro->	1615	-	0.1	-	-
56	*β*-Cedene epoxyde	1637	-	0.1	-	-
57	Selin-11-en-4α-ol	1970	-	-	0.6	-
58	Total %		99.8	98.5	97.9	97.1

Legend: LRI: Linear Retention Indice; P.b: *Pentadiplandra brazzeana*; M.p: *Mentha sp cf piperita*; E.c: *Eugenia caryophylla*; C.c: *Cymbopogon citratus*.

Table No.6: Results of sensitivity tests of essential oils on certain microbial isolates

S.No	Microbial isolates	Gentamicin®	*P. brazzeana*	*E. caryophylla*	*C. citratus*	*M. sp cf piperita*
1	*Bacillus cereus*	31.5 ± 0.28^{b}	$35.5 \pm 0.28^{a,b,c,d}$	10.5 ± 0.28^{a}	13.5 ± 0.28^{b}	0 ± 0^{a}
2	*Bacillus subtilis*	$30.5 \pm 0.28^{a,b}$	$25 \pm 2.88^{a,b,c}$	12.5 ± 1.4^{a}	14.5 ± 2.02^{b}	$4 \pm 2.30^{a,b}$
3	*Enterobacter cloacae*	28.5 ± 0.86^{a}	$39.5 \pm 6.06^{b,c,d}$	12 ± 0.57^{a}	10.5 ± 0.28^{b}	$8 \pm 0.57^{b,c}$
4	*Klebsiella oxytoca*	40 ± 0.57^{c}	$24 \pm 0.57^{a,b}$	11.5 ± 0.28^{a}	0 ± 0^{a}	0 ± 0^{a}
5	*Klebsiella pneumoniae*	32 ± 1.15^{b}	$42 \pm 6.92^{c,d}$	11.5 ± 0.28^{a}	20 ± 0.57^{c}	$14.5 \pm 1.44^{d,e}$
6	*Staphylococcus aureus*	32.5 ± 1.44^{b}	43.5 ± 3.17^{d}	9.5 ± 0.28^{a}	0 ± 0^{a}	0 ± 0^{a}
7	*Streptococcus* spp	32 ± 1.15^{b}	20 ± 0.57^{a}	9.5 ± 0.28^{a}	14.5 ± 0.86^{b}	16 ± 1.15^{e}

Legend: The values that had the same letters were statistically identical while those with different letters were statistically different with a significance level $P < 0.05$.

Table No.7: Inhibition parameter of essential oils

			Essential oils				Antibiotic
S.No	**Microbial isolates**	**Parameters (mg/ mL)**	***C. citratus***	***E. caryophylla***	***M. sp cf piperita***	***P. brazzeana***	**Gentamicin® (µg/mL)**
1	*Bacillus cereus*	CMI	/	0.31	0.62	0.31	0.25
		CMB	/	0.62	ND	0.62	0.25
		CMB/CMI	/	2	ND	2	1
2	*Basillus suptilus*	CMI	1.25	0.62	0.62	0.31	0.25
		CMB	ND	1.25	ND	0.62	0.25
		CMB/CMI	ND	2	ND	2	1
3	*Enterobacter cloacae*	CMI	/	1.25	/	0.62	0.12
		CMB	/	1.25	/	1.25	0.25
		CMB/CMI	/	1	/	2	2
4	*Klebsiella oxytoca*	CMI	1.25	0.62	1.25	0.62	0.12
		CMB	ND	ND	ND	ND	0.25
		CMB/CMI	ND	ND	ND	ND	2
5	*Klebsiella pneumoniae*	CMI	0.62	0.07	0.62	0.15	0.25
		CMB	0.62	0.07	0.62	0.31	0.25
		CMB/CMI	1	1	1	2	1
6	*Staphylococcus aureus*	CMI	1.25	0.62	/	0.31	0.12
		CMB	ND	1.25	/	1.25	0.25
		CMB/CMI	ND	2	/	4	2
7	*Streptococcus* spp.	CMI	0.62	0.31	1.25	0.15	0.12
		CMB/CMI	1	1	1	1	1

ND: Not Determined; P.b: *Pentadiplandra brazzeana*; M.p: *Mentha sp cf piperita*; E.c: *Eugenia caryophylla*; C.c: *Cymbopogon citratus*.

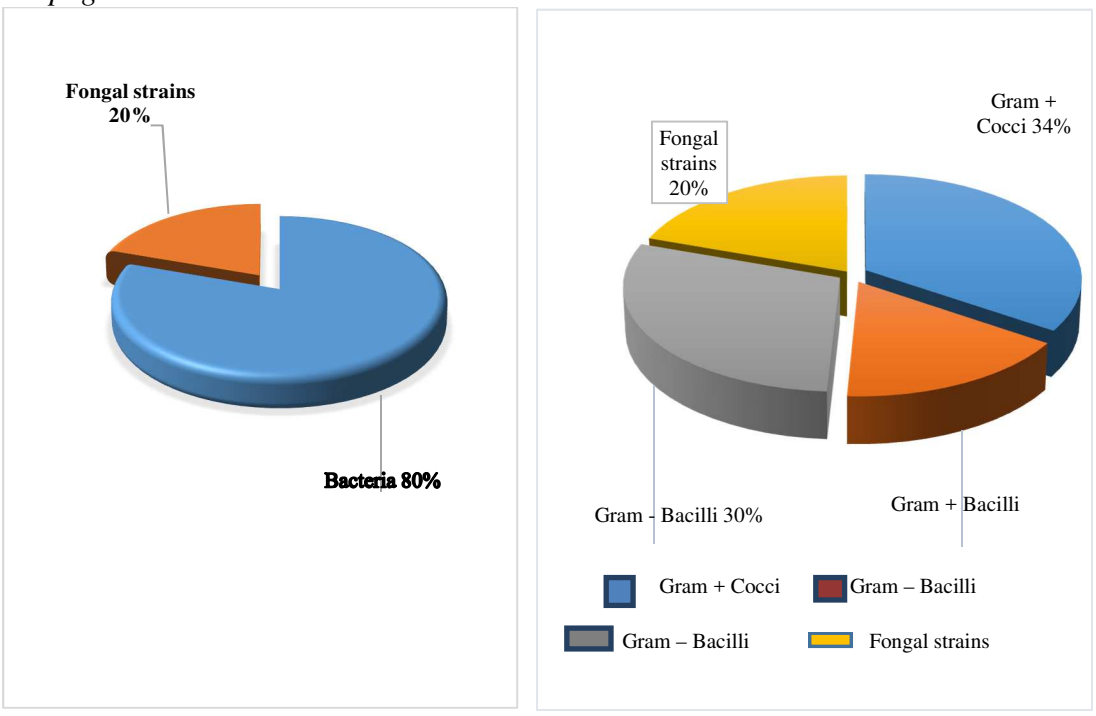

Figure No.1: A = Percentage of isolated microbial types (bacteria or yeasts); B = Distribution of bacterial isolates according to their Gram type

CONCLUSION

At the end of this study whose general objective was to characterize the germs responsible for oral infections, but also to enhance the antimicrobial potential of essential oils derived from certain Cameroonian medicinal plants on some identified microbial isolates. The results of this study revealed that, many microorganisms are found in the mouth like *Bacillus cereus, Klepsiela pnemoniea, Streptococcus spp* and *Staphilococcus aureus*. Essentials oils were found to have antibacterial and antifungal activities against the isolated germ, and may be suggested as a new potential source of natural antimicrobial for the prevention and control of bacterial infections of oral cavity. Nevertheless further research is still needed in order to determine if they could efficiently substitute the synthetic antibiotics or used in combination.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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