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Chemical Characterization and Antibacterial Efficacy of Essential Oils of Three Lamiaceae Species Growing in Cameroon

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Abstract: Essential oils (EOs) have aroused attention among the naturally-occurring therapeutic compounds with anti-infective properties. This study examined the chemical composition and the antibacterial potential of EOs from three Lamiaceae species including *Ocimum gratissimum* (leaves), *Plecthrantus epilithicus* (leaves), and *Satureja robusta* (leaves and flowers). EOs were obtained by hydrodistillation using a Clevenger-type apparatus, followed by characterization by gas chromatography-mass spectrometry (GC-MS) and flame ionization detector (GC/FID). The antibacterial efficacy of EOs was screened using the microdilution method, against a panel of eight foodborne bacteria isolates namely *Enterobacter cloacae*, *Yersinia enterocolotica*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Escherichia coli*, *Citrobacter freundii*, *Shigella flexneri*, and *Enterococcus faecalis*. GC-MS and GC/FID analysis led to the identification of 53 components from EOs of *P. epilithicus*, while around forty compounds have been characterized from the EOs of *O. gratissimum* (43), leaves of *Satureja robusta* (43), and flowers of *S. robusta* (44). The nature of identified compounds varied according to the species. The most prevalent volatile compounds identified in the EOs of *P. epilithicus* were piperitenone oxide (23.65%) and two isomers piperitone oxide 2/2 (16.15%) and piperitone oxide 1/2 (7.24%). The major constituents in the EOs of leaves of *O. gratissimum* were elemicin (33.474%) and eugenol (30.258%). Piperitone oxide, piperitone, and germacrene D were identified as major constituents in both the EOs of leaves of *S. robusta* (28.3%, 15.14%, and 9.42% respectively) and flowers (45.6%, 11.55%, and 3.94% respectively). The oils displayed selective antibacterial potential, with the recorded minimal inhibitory concentration (MIC) values ranging from 0.0156 to 1% (v/v). EOs of *P. epilithicus* as well as that of the leaves and flowers of *S. robusta* acted against all studied bacteria. Most of the MIC values were below 0.25% (v/v), indicating a strong inhibitory potential of studied EOs. The present study provides a strong baseline for consideration of the EOs from *O. gratissimum*, *S. robusta*, and *P. epilithicus* in the control of bacterial foodborne infections.

Keywords: Essential Oils, Chemical Composition, Antibacterial Activity, Foodborne Bacteria, *O. gratissimum*, *S. robusta*, *P. epilithicus*

1. Introduction

Numerous investigations showed evidence of the pharmacological properties of medicinal plants and derived products. Indeed, there is an urgent need for alternative medicine due to the upsurge of many diseases resistant to traditional treatments, including infectious diseases. Most of the commonly used antibiotics have lost their effectiveness, due to the multiple drug resistance (MDR) developed by pathogenic bacteria [1]. Another problem facing modern medicine is the toxicity of commonly used synthetic therapies. This explains the rush towards naturally-occurring chemotherapeutic agents, supposedly less toxic. Plants usually produce many molecules called secondary metabolites intended to protect them against the harmful effects of their environment as well as possible diseases; which properties could be transferred to humans for disease controls. Essential oils (EOs) are among the many substances produced by plants, with substantial biological properties. Essential oils are complex mixtures of hydrocarbons and oxygenated hydrocarbons biosynthesizing from the isoprenoid pathways, mainly made of monoterpenes and sesquiterpenes [2]. Regarding the properties of essential oils, they are oily, hydrophobic, aromatic, and volatile liquids or semi-liquids, extracted from plants, usually by steam distillation [3]. Essential oils may be derived from specialized cells or groups within particular regions of the plant, such as stems, leaves, the foliage, bark, wood, fruit, seeds, and rhizomes. During several civilizations, they have been exploited worldwide in folk medicine, owing to their pharmacological properties [4]. Plant essential oils have aroused attention among the naturally-occurring therapeutic agents with anti-infective activity. Several among these secondary plant metabolites exhibit marked antimicrobial effects that have made their use as an antiseptic and/or preservative in food well known, since antiquity [5]. In addition to their antimicrobial properties, numerous investigations documented the therapeutic uses of essential oils including anti-parasitic, analgesic, antioxidant, anti-inflammatory, anti-obesity, anticancer, wound-healing, antispasmodic, allelochemicals properties, and many more [2, 6]. Relative modes of action have been unveiled along with pharmacological targets, though the shortage of human studies restraints the potential of essential as efficient and safe phytotherapeutic agents [6].

The chemical composition of essential oils is influenced by exogenous and endogenous factors, leading to ecotypes or chemotypes in the same plant species. The endogenous factors are allied to anatomical and functional characteristics of the plants and to the biosynthetic pathways of the volatiles, which might change in either the different tissues of the plants or in different seasons, but also could be influenced by DNA adaptation. The exogenous factors (such as light, precipitation, season, altitude, and soil characteristics), over a long period, might affect some of the genes responsible for volatiles formation [7]. The antibacterial activities of essential oils are related to their chemical composition, the proportions of volatile molecules, and their interactions [8]. Some major essential oils constituents namely thymol, eugenol, and carvacrol displayed interesting antimicrobial effects towards a wide spectrum of bacteria comprising *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella enterica*, *Clostridium jejuni*, and *Staphylococcus aureus* [9]. The bacteria listed are among the major foodborne pathogens causing foodborne illnesses [10]. This suggests the potential of essential oils and derived components against foodborne bacteria. Evenly, other families of essential oil constituents also have noteworthy antibacterial effects; these include alcohols, aldehydes, ketones, monoterpenes (examples of geraniol, linalol, menthol, terpineol, thujanol, myrcenol, citronellal, neral, thujone, camphor, carvone, γ-terpinene, *p*-cymene, among others), and phenylpropanes (cinnamaldehyde). The Lamiaceae family has been described to be a rich source of essential oils [11]. Essential oils from the Lamiaceae plants have been widely documented to possess marked pharmacological potential. Some examples include rosemary (*Rosmarinus officinalis L.),* oregano (*Origanum vulgare*), and thyme (*Thymus sp.*) which have great potential as food preservatives due to their notable antioxidant and antimicrobial activities [12]. Our study focused on three Lamiaceae plant species growing in Cameroon, namely *Ocimum gratissimum*, *Satureja robusta*, and *Plecthrantus epilithicus*.

Ocimum gratissimum is commonly used as spices, especially flowers and leaves. It is used in traditional medicine as sedative (decoction of roots), treatment of epilepsy, fever, diarrhea, management of mental illness (decoction of leaves), fungal infections, cold, catarrh, blocked nostrils, abdominal pains, sore eyes, ear infections, coughs, barrenness, tooth gargle, sunstroke, headache, diaphoresis, inflammation, stomach upset, haemorrhoids, diarrhoea, pneumonia, cough, conjunctivitis [13]. The major constituents present in *O. gratissimum* essential oils involve eugenol, methyl eugenol, thymol, cis-ocimene, trans-ocimene, α-pinene, camphor [14-17]. Studies highlighted the antibacterial properties of essential oils from *O. gratissimum* against *Staphylococcus aureus*, *Escherichia coli*, *Serratia marcescens*, *Aspergillus niger*, *Streptococcus faecalis, Pseudomonas aeruginosa,Shigella sp.* [17, 18]; the antibiotic resistance-modifying activities [19], as well as the antifungal and antioxidant properties [17].

Satureja robusta is traditionally used to relieve muscle pain, as a tonic and carminative agents, to treat stomach and intestinal disorders (cramps, nausea, indigestion, and diarrhea)

[20]. A previous study done by Tchoumbougnang et al. [21] concluded that menthone, geraniol, thymol, germacrene D are major constituents of *S. robusta* essential oils. The *S. robusta* essential oils showed antifungal activity against *Aspergillus niger* [21].

Plecthrantus epilithicus is used in folk medicine to manage digestive, skin, infective and respiratory problems [22]. To the best of our knowledge, no chemical composition as well as pharmacological activities of *P. epilithicus* essential oils has been reported so far in the literature.

The essential oils from the abovementioned Lamiaceae plant species have been investigated for their antibacterial effectiveness against foodborne bacteria in the present study. The gas chromatography-mass spectrometry (GC-MS) coupled to a flame ionization detector (GC/FID) analysis were applied for their chemical characterization.

2. Materials and Methods

2.1. Plant Material, Collection and Authentication

The plants of interest included three Lamiaceae species growing in Cameroon. *Satureja robusta* (leaves and flowers) and *Plecthrantus epilithicus* (leaves) were harvested in West and Southwest Regions of Cameroon in July 2018, whereas *Ocimum gratissimum* (leaves) has been collected at the Haut-Nkam Division (West Region-Cameroon) in January 2018. The plant parts have been carefully identified and authenticated at the National Herbarium of Cameroon (HNC) where voucher specimens were deposited under registration numbers (*O. gratissimum* 23798HNC, *P. epilithicus* 9729HNC, and *S. robusta* 12777 SFRCAM).

2.2. Extraction of Essential Oils

The essential oils of the three Lamiaceae species were obtained following hydrodistillation with Clevenger-type apparatus. A mass of 150 g of dried plant material was introduced into a 4-liter flask, then 2.5 liters of water were added and the whole was brought to the boil for 5 hours. The water residues were removed from essential oils collected at the end of the distillation with anhydrous sodium sulfate (Thomas Baker Chemicals, Mumbai, India). The extraction yield has been calculated according to the following equation.

$$\text{Yield (\%)} = \text{Mass of extracted oil} / \text{Mass of dry vegetal}$$

The essential oils obtained were then stored at 4°C in a smoked bottle for further uses.

2.3. GC-MS and GC/FID Analysis of the Chemical Profile of Essential Oils

The essential oils from each plant were analyzed in the apolar mode. Two signals have been recorded corresponding to mass spectrometry (MS) and the flame ionization detector (FID) allowing respectively the identification and quantification of the detected compounds.

An Agilent Technologies chromatographer (model 7890A) coupled to an Agilent 5975C mass selective detector (MSD) was used. The system was controlled by ChemStation software. The injection volume of the essential oils was 0.2 µL under a 150:1 split ratio. Two DB-1 columns (30 m x 250 µm x 0.25 µm, stationary phase film of dimethylpolysiloxane type) were used. The oven operating conditions were: initial temperature 50°C for 2 min, then rising from 50°C to 150°C at 7.5°C/ min for 3 min and finally kept isothermal at 250°C for 16 min before post-run (250°C for 5 min). Helium was used as carrier gas at 1.2 mL/min. The injection and transfer line temperatures were 150°C and 250°C, respectively. The detector temperature was maintained at 250°C, the flow of H_2 at 40 mL/min, and the flow of air at 400 mL/min. Mass detection was carried out in scanning mode between 32 and 450 Daltons.

The apolar retention indices (RI), as well as the mass spectra, were compared with those compiled in the National Institute of Standards and Technology (NIST 14) library for compounds identification.

2.4. Antibacterial Efficacy Investigation

2.4.1. Selected Bacteria

The study involved eight bacteria isolates, including *Enterobacter cloacae*, *Yersinia enterocolotica*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Escherichia coli*, *Citrobacter freundii*, *Shigella flexneri*, and *Enterococcus faecalis*. The bacteria were from a laboratory collection isolated from fishes. The studied bacteria are commonly involved in foodborne infections [10]. Mueller Hinton Agar (MHB) and Mueller Hinton Broth (MHB) were used as culture media for antibacterial testing. MHA and MHB are recommended by CLSI for antibacterial susceptibility testing. They are non-selective, non-differential medium, allowing almost all microorganisms to grow. Before any experiment, studied bacteria were subcultured (37°C, 18–24 h) in MHA. MHB was used for microdilution. Bacteria inoculum was initially prepared in sterile distilled water, the turbidity adjusted with a spectrophotometer to a McFarland standard of 0.5, equivalent to 1.5×10^8 CFU/mL.

2.4.2. Minimum Inhibitory and Minimum Bactericidal Concentrations Determination

The antibacterial testing of essential oils was carried out using the 96-well microplates broth microdilution technique. The iodonitrotetrazolium (INT, Merck, Germany) served as the bacterial growth indicator. The test was based on previously described protocols [23-25]. Briefly, essential oils and a reference antibiotic (ciprofloxacin) were dissolved in Tween 80/MHB to obtain the working solution. The final concentration of Tween 80 in the assay was less than 2.5%, a concentration innocuous to bacterial growth. The solution obtained was added to MHB, followed by a two-fold serial dilution in a 96-wells microplate. Subsequently, bacterial suspension initially prepared at the McFarland standard of 0.5 (1.5×10^8 CFU/mL), as above mentioned, was diluted in MHB, and 100 µL of bacterial inoculum was seeded in the wells of plates containing test samples. The final inoculum

concentration was equivalent to 1.5 × 10^6 CFU/mL. Afterward, the plates were covered with a sterile plate sealer, followed by incubation at 37°C for 18 h. Wells containing adequate MHB, 100 µL of inoculum, and Tween 80 to a final concentration of 2.5% served as negative controls. The final concentration of the essential oils varied from 0.0078 to 1% (v/v), whereas that of antibiotics ranged from 0.25 to 32 µg/mL. The MIC of test samples was recorded after 18 h incubation at 37°C, following the addition (40 µL) of INT 0.02% (w/v) and incubation at 37°C for 30 min. The viable bacteria reduced the yellow dye to pink. Wells with test samples only (blank control) were used to ensure that the essential oils were not producing color with INT.

The MBC was assessed by adding 50 µL aliquots of the preparations, which did not show any growth after incubation during MIC testing, to 150 µL of adequate broth (MHB). These preparations were incubated at 37°C for 48 h. The MBC was considered as the lowest concentration of samples that prevented the color change of the medium after the addition of INT as abovementioned. The assays (for both MIC and MBC) were performed in triplicate and repeated thrice.

3. Results and Discussion

3.1. Chemical Composition of Essential Oils

The highest extraction yield of essential oils (EOs) was obtained with the leaves of *O. gratissimum* (0.42%), followed by the flowers of *S. robustta* (0.40%), leaves of *P. epilipthicus* (0.35%), and the leaves of *S. robusta* (0.30%). The identified compounds in the EOs of the studied plant species along with their percentage composition and retention index (RI) were summarized in Table 1.

Table 1. *Chemical composition of essential oils of O. gratissimum (leaves), P. epilithicus (leaves), and S. robusta (leaves and flowers) on the apolar column.*

Identified compounds	FID: % of identified compounds					
	PE	SB (leaves)	SB (flowers)	OG		
HYDROGENATEDMONOTERPENES	6.525	2.618	2.261	13.723	CAS number	FID: RI
Alpha pinene	0.099	0.255	0.329	/	80-56-8	930
Camphene	0.037	/	/	/	79-92-5	943
Beta pinene	/	0.431	0.484	/	127-91-3	970
Sabinene	/	0.226	0.262	/	3387-41-5	970
Delta-3-carene	1.495	/	/	/	13466-78-9	1005
Myrcene	0.55	0.2	0.316	0.25	123-35-3	983
Alpha phellandrene	0.11	/	/	/	99-83-2	996
Alpha terpinene	0.123	/	/	/	99-86-5	1009
Sylvestrene	0.085	/	/	/	1461-27-4	1018
Limonene	0.86	0.143	0.2	/	138-86-3	1022
Beta phellandrene		0.01	0.012	/	555-10-2	1020
(Z) ocimene	0.066	0.12	0.059	**10.329**	3338-55-4	1027
(E) ocimene	0.129	1.186	0.569	2.941	3779-61-1	1038
P-cymene	0.819	0.047	0.03	0.011	99-87-6	1012
Terpinolene	1.926	/	/	/	586-62-9	1080
4,8-dimethyl-1,3,7-nonatriene	/	/	/	0.041	51911-82-1	1106
Allo ocimene ½	/	/	/	0.151	673-84-7_1	1119
Dehydro p-cymene	0.226	/	/		1195-32-0	1074
OXYGENATED MONOTERPENES	59.927	51.509	66.901	0.066	CAS number	FID: RI
Eucalyptol	/	0.056	0.05	/	470-82-6	1020
Menthone	/	1.442	0.088	/	89-80-5	1144
Isomenthone	/		**3.368**	/	491-07-6	1144
Linalool	/	0.599	0.711	0.066	78-70-6	1087
Cis p-menth-2-en-1-ol	0.117	/	/	/	29803-82-5	1108
Neomenthyl acetate	/	2.196	1.329	/	2230-87-7	1281
Isopulegol acetate	/	0.087	/	/	89-49-6	1259
Menthyl acetate	/	0.409	/	/	16409-45-3	1294
Neoisomenthol	/	/	0.283	/	89-78-1	1171
Isomenthol	/	0.325	0.783	/	490-99-3	1176
Pulegone	/	/	1.098	/	89-82-7	1216
Myrtenyl acetate	/	0.125	/	/	1079-01-2	1307
Borneol	0.111	/	/	/	507-70-0	1152
Piperitone	**4.614**	**15.139**	**11.55**	/	89-81-6	1234
Diosphenol	0.064	/	/	/	490-03-9	1279
P-cymen-8-ol	1.643	/	/	/	1197-01-9	1164
Isopiperitenone	/	0.081	0.108	/	529-01-1	1236
Piperitenone	1.343	1.083	1.933	/	491-09-8	1311
Oxyde piperitone 1/2	**7.236**	**28.3**	**45.6**	/	5286-38-4	1234
Oxyde piperitone 2/2	**16.149**	/	/	/	20303-83-7	1234
Oxyde piperitenone	**28.65**	0.899	/	/	35178-55-3	1340
Neomenthol		0.264	/	/	3623-51-6	1171
Menthol		0.504	/	/	89-78-1	1176

Identified compounds	FID: % of identified compounds					
	PE	**SB (leaves)**	**SB (flowers)**	**OG**		
HYDROGENATEDSESQUITERPENES	13.017	16.098	7.975	15.876	CAS number	FID: RI
Alpha cubebene	0.117	/	/	0.042	17699-14-8	1349
Alpha copaene	2.189	0.6	0.37	0.581	3856-25-5	1376
Beta bourbonene	0.965	2.362	1.051	0.181	5208-59-3	1384
Alpha gurjunene ½	/	/	0.101	/	489-40-7_1	1409
Beta cubebene	0.474	0.45	0.216	0.367	13744-15-5	1387
Trans alpha bergamotene	/	/	/	0.037	13474-59-4	1410
Trans beta copaene	0.13	0.421	0.158	/	20479-06-5	1417
Beta elemene	0.568	0.552	0.574	0.127	515-13-9	1387
Caryophyllene	1.375	1.64	1.162	**3.13**	87-44-5	1416
(6E) beta farnesene	/	/	/	0.214	18794-84-8	1447
Allo aromadendrene	/	/	/	0.145	25246-27-9	1454
Alpha humulene	0.404	/	0.05	0.274	6753-98-6	1446
Gamma selinene	0.201	0.115	/	/	515-17-3	1474
Germacrene D	**4.213**	**9.42**	**3.944**	**3.189**	23986-74-5	1474
Beta bisabolene	/	/	/	**5.601**	495-61-4	1504
(3E, 6E) alpha farnesene	/	/	/	1.452	502-61-4	1497
Bicyclogermacrene	/	/	/	0.306	67650-90-2	1490
Delta cadinene	1.04	0.439	0.269	0.23	483-76-1	1518
Gamma cadinene	0.203	0.099	0.08	/	39029-41-9	1486
7-epi-alpha selinene	0.45	/	/	/	123123-37-5	1516
Cis calamenene	0.066	/	/	/	483-77-2	1512
Gamma calacorene	0.063	/	/	/	24048-45-1	1534
Alpha selinene	0.353	/	/	/	473-13-2	1474
Beta selinene	0.206	/	/	/	515-17-3	1479
OXYGENATED SESQUITERPENES	5.595	1.605	1.852	1.663	CAS number	FID: RI
Cis Muurol-5-En-4-Alpha-ol	0.53	0.216	/	0.111	81967-90-0_1	1486
Trans muurol-5-en-4-alpha-ol	0.442	0.056	0.06	0.11	81967-90-0_2	1509
Isocaryophyllene-5,6-epoxyde		0.126	/	0.167	17627-43-9	1576
Oxyde caryophyllene	0.438	0.349	0.418	0.694	1139-30-6	1569
(E) nerolidol	0.297	/	/	0.092	40716-66-3	1555
Alpha humulene oxide	/	/	/	0.046	19888-34-7	1606
1 (10)-germacradien-4-alpha-ol	/	/	0.09	/	72120-50-4	1575
Spathulenol	/	/	/	0.333	6750-60-3	1573
Guaia-6,9-dien-4-beta-ol	/	/	0.186	0.11	1105692-17-8	1572
Tau cadinol	0.233	0.211	0.2	/	01/11/5937	1630
Delta cadinol	0.196	/	0.126	/	19435-97-3	1638
Alpha cadinol	1.316	0.647	0.772	/	481-34-5	1642
Neointermedeol	1.919	/	/	/	5945-72-2	1647
Alismol	0.224	/	/	/	87827-55-2	1613
PHENYLPROPANOIDS	1.365	0.32	0.269	63.832	CAS number	FID: RI
Benzaldehyde	0.036	/	/	0.018	100-52-7	929
Methyl eugenol	/	/	/	0.072	93-15-2	1376
Eugenol	0.17	/	/	**30.258**	97-53-0	1339
Thymol	1.159	0.32	0.269	/	89-83-8	
Elemicin	/	/	/	**33.474**	487-11-6	1535
Indol	/	/	/	0.005	120-72-9	1262
Vanillin	/	/	/	0.005	121-33-5	1358
OTHERS	0.125	2.596	2.291	0.412	CAS number	FID: RI
(2E)-hexenal	/	/	/	0.063	6728-26-3	825
3-octyl acetate	/	2.4	1.776	/	4864-61-3	1110
Methyl heptenone	/	/	/	0.036	110-93-0	964
Hexyl alcohol	/	/	/	0.012	111-27-3	853
Methyl heptenone	0.055	/	/	/	110-93-0	963
Ethyl amyl carbinol	/	0.196	0.369	/	589-98-0	983
(2E)-hexenol	/	/	/	0.034	928-95-0	850
1-octen-3-ol	0.07	/	/	0.199	3391-86-4	966
Acetic acid	/	/	0.146	/	64-19-7	576
(3Z)-hexenol	/	/	/	0.025	928-96-1	839
2-methyl 2,4-heptadien-6-one	/	/	/	0.043	1604-28-0	1081

*FID: Flame Ionization Detector. RI: Retention Index. CAS: Chemical Abstracts Service. Values in bold: Major constituents. OG: *O. gratissimum*. PE: *P. epilithicus*. SB: *S. robusta*.

Volatile constituents identified in the *O. gratissimum* EOs were 6 hydrogenated monoterpenes (13.723%), 1 oxygenated monoterpene (0.066%), 15 hydrogenated sesquiterpenes (15.876%), 8 oxygenated sesquiterpenes (1.663%), 6

phenylpropanoids (63.832%), and 7 other components (0.412%) comprising 43 constituents (95.57%) of the total oil. Quantitatively, the major constituents were elemicin (33.474%) and eugenol (30.258%). The other minor compounds were (Z) ocimene (10.329), beta bisabolene (5.601%), germacrene D (3.189%), and caryophyllene (3.13%). The present study reports for the first time the presence of elemicin as the major constituent in *O. gratissimum* oils. Previous investigations on the EOs of *O. gratissimum* have reported the presence of eugenol as the major component [17]. This study is consistent with previous findings. The minor compounds identified have also been documented in previous studies on this plant [14-17], in different proportions (percentages). Similar to the present study, Joshi [17] also showed that the oxygenated monoterpenes were the least class compounds found in *O. gratissimum* EOs.

The most prevalent volatile compounds identified in the EOs of *P. epilipthicus* were piperitenone oxide (23.65%) and two isomers piperitone oxide 2/2 (16.15%) and piperitone oxide 1/2 (7.24%). The other minor compounds were piperitone (4.614%) and germacrene D (4.213%). The class compositions were 13 hydrogenated monoterpenes (6.525%), 9 oxygenated monoterpenes (59.927%), 17 hydrogenated sesquiterpenes (13.017%), 9 oxygenated sesquiterpenes (5.595%), 3 phenylpropanoids (1.365%), and 2 other components (0.125%) comprising 53 constituents (86.55%) of the total oil. To the best of our knowledge, the present investigation report for the first time the chemical composition of *P. epilipthicus* EOs.

The class compositions of the leaves of *S. robusta* EOs were 9 hydrogenated monoterpenes (2.618%), 15 oxygenated monoterpenes (51.509%), 10 hydrogenated sesquiterpenes (16.098%), 6 oxygenated sesquiterpenes (1.605%), 1 phenylpropanoid (0.32%), and 2 other components (2.596%) comprising 43 constituents (74.75%) of the total oil. Constituents identified in the EOs of *S. robusta* flowers were 9 hydrogenated monoterpenes (2.261%), 12 oxygenated monoterpenes (66.901%), 11 hydrogenated sesquiterpenes (7.975%), 7 oxygenated sesquiterpenes (1.852%), 1 phenylpropanoid (0.269%), and 3 other components (2.291%) comprising 44 constituents (81.80%) of the total oil. Piperitone oxide, piperitone, and germacrene D were identified as major constituents in both the leaves (28.3%, 15.14%, and 9.42% respectively) and flowers (45.6%, 11.55%, and 3.94% respectively) of *S. robusta* EOs, at different percentages. Another prevalent component found in *S. robusta* flowers was isomenthone (3.37%). Investigations by Tchoumbougnang et al. [21] reported the presence of menthone, geraniol, thymol, and germacrene D as major constituents of *S. robusta* harvested in the Northwest Region (Bamenda) of Cameroon. The discrepancy is notable with our findings. Eleven compounds were found to be common in the studied EOs, which were myrcene, ocimene, *p*-cymene, alpha copaene, beta bourbonene, beta elemene, caryophyllene, germacrene D, delta cadinene, trans murol-5-en-4-alpha-ol, oxide caryophyllene in less quantity.

The chemical composition of EOs depends on the harvesting area of the plant, the climate, and the type of soil where the species are grown [26]. These characteristics would also influence biological activities since major compounds could be altered [27]. The chemical composition of EOs is influenced by exogenous and endogenous factors. The endogenous factors are related to anatomical and physiological characteristics of the plants and to the biosynthetic pathways of the volatiles, which might change in either the different tissues of the plants or in different seasons, but also could be influenced by DNA adaptation. The exogenous factors, over a long period, might affect some of the genes responsible for volatiles formation. Those factors lead to ecotypes or chemotypes in the same plant species [7]. This would justify the variability in the chemical composition of the different studied species of the Lamiaceae family, as well as the difference in chemical composition between the parts of the same plant.

3.2. Antibacterial Activities of Essential Oils

Essential oils (EOs) of *P. epilithicus*, *S. robusta*, and *O. gratissimum* were tested on Gram-negative bacteria *Enterobacter cloacae*, *Yersinia enterocolotica*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Escherichia coli*, *Citrobacter freundii*, *Shigella flexneri*, and a Gram-positive bacteria *Enterococcus faecalis*. The results of the antibacterial testing of studied EOs are presented in Table 2. The MIC and MBC are represented in % (v/v). The recorded MIC values ranged from 0.0156 to 1% (v/v). EOs from the leaves of *P. epilithicus*, as well as leaves and flowers of *S. robusta* acted against all studied bacteria. Most of the MIC values were below 0.25% (v/v), indicating the interesting and strong inhibitory potential of studied EOs. MIC ≤ 0.25% (v/v) were obtained with EOs of *P. epilithicus* leaves, as well as leaves and flowers of *S. robusta* against 7 out of 8 studied bacteria isolates, while EOs of *O. gratissimum* displayed similar values against 6 bacteria. The lowest MIC value was obtained with EOs from the flowers of *S. robusta* and *O. gratissimum* leaves (MIC=0.0156%) against *E. coli*. This indicates the significant antibacterial potential of test oils against *E. coli*. Bactericidal effects were obtained with oils from leaves of *P. epilithicus* and flowers of *S. robusta* against *C. freundii*, oils from *S. robusta* leaves against *K. pneumoniae*, and oils from *O. gratissimum* leaves against *E. coli* and *S. flexneri*. Previous findings also documented on the interesting antimicrobial activities against bacteria and fungi [17, 18], as well as antibiotic resistance-modifying activities [19] of *O. gratissimum*. This is consistent with the data obtained in this study. The antifungal action of *S. robusta* EOs against *A. niger* has been reported [21]. The present study also demonstrates its antibacterial potential. To the best of our knowledge, no investigations have been reported on the antimicrobial activities of *P. epilithicus* EOs. Therefore, the present work provides information regarding its antibacterial properties. The tested bacteria were isolated from fishes. These bacteria are generally involved in foodborne infections, which are serious public health

concerns. Indeed, foodborne pathogens are causing a great number of diseases with significant effects on human health and the economy [10]. The marked activities of the test EOs from this study provide important and relevant baselines for their use in the control of foodborne infections.

The variability of the chemical composition and in particular the major compounds identified in the investigated oils would justify their remarkable antibacterial potential. The major compounds would act alone or in interaction with other compounds present in the mixture. Elemicin and eugenol, the two major constituents from *O. gratissimum* are well known to possess interesting antimicrobial potential. Rossi et al. [28] displayed the antibacterial activity of elemicin against the human enteropathogen *Campylobacter jejuni*. Besides, eugenol has shown significant broad-spectrum antimicrobial activities against Gram-positive, Gram-negative, fungi, and virus. Eugenol has also shown synergistic effects with conventional antimicrobials [29]. Documented investigations have demonstrated antibacterial activities of EOs rich in piperitenone oxide and piperitone (major constituents of the *P. epithilicus* and *S. robusta* oils obtained in the present work) [30]. The presence of these compounds may account for the recorded activities.

Table 2. *Minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of studied essential oils.*

Bacteria	MIC and MBC of essential oils* from studied plants								ATB
	P. epilithicus **(leaves)**		***S. robusta*** **(leaves)**		***S. robusta*** **(flowers)**		***O. gratissimum*** **(leaves)**		**CIP**
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**
Enterobacter cloacae	0.0625	>1	0.125	>1	0.125	>1	0.25	0.25	0.25
Yersinia enterocolitica	0.25	>1	0.125	>1	0.25	>1	>1	>1	0.5
Klebsiella pneumoniae	0.0625		0.125	0.25[b]	0.0625	0.5	0.0312	>1	0.5
Salmonella typhi	0.0625	>1	0.125	>1	0.125	>1	0.125	>1	0.25
Escherichia coli	0.125	>1	0.0312	0.5	0.0156	0.5	0.0156	0.0312[b]	0.25
Citrobacter freundii	0.0625	0.125[b]	0.125	1	0.0312	0.125[b]	0.125	>1	0.25
Shigella flexneri	0.0312	0.5	0.125	>1	0.0625	0.5	0.25	1[b]	1
Enterococcus faecalis	1	>1	1	>1	1	>1	>1	>1	0.5

*Essential oils tested at 1% (v/v) and antibiotic tested at 32 µg/mL. MIC: Minimum Inhibitory Concentrations. MBC: Minimum Bactericidal Concentrations. [b] Bactericidal activities. ATB: Antibiotic. CIP: Ciprofloxacin.

P. epithilicus showed the highest antibacterial activity with 53 compounds identified, followed by the flowers of *S. robusta* (44 compounds), the leaves of *S. robusta* (43 compounds), and the leaves of *O. gratissimum* (43 compounds). A correlation could also be noted between the significant antibacterial effectiveness of *P. epithilicus* and *S. robusta* oils and their high content of oxygenated monoterpenes. Furthermore, the high phenylpropanoid content of *O. gratissimum* oils could explain the activities noted towards the foodborne bacteria tested. These findings agreed with previous investigations, since it has been demonstrated that antibacterial activity of essential oils related to their chemical composition, the proportions of volatile molecules, and their interactions [8].

4. Conclusion

The present work displayed the chemical composition and antibacterial potential of essential oils of *P. epithilicus*, *S. robusta*, and *O. gratissimum*. All studied essential oils depicted noteworthy antibacterial efficacy against foodborne bacteria tested, *P. epithilicus* appeared as the most active, followed by flowers of *S. robusta*, leaves of *S. robusta*, and finally the leaves of *O. gratissimum*. The major constituents found from these Lamiaceae were piperitone oxide, piperitone, elemicin, and eugenol. The present study provides a strong baseline for consideration of studied essential oils in the management of bacterial infections and particularly foodborne infections.

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