

Chemical Composition, Antioxidant and Antimicrobial Activity of Essential Oils Isolated from Two Piper Species Collected in Comoros

Soidrou S H^{1,2*}, Farah A², Satrani B³, Ghanmi M³, Lachkar M⁴, Ahamada Himidi A¹

¹Laboratory of Foods and Natural Substances Reactivity and Synthesis, Faculty of Sciences and technology, University of Comoros, BP 2585, Moroni, Comoros

²Laboratory of Applied Organic Chemistry, Faculty of Sciences and technology Saïss, Sidi Mohamed Ben Abdallah University, Po. Box 2202. Fez, Morocco

³Centre de recherche forestière, BP.763, 10050, Rabat, Agdal, Morocco

⁴Engineering Laboratory of Organometallic and Molecular Materials, CNRST (URAC 19), Faculty of Sciences, University Sidi Mohamed Ben Abdallah, Po. Box 1796 (Atlas), 30000 Fez, Morocco

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ABSTRACT

The volatile composition of two piper species growing in Comoros was investigated in this study. The essential oils were obtained by hydrodistillation and their analyses were performed by GC and GC/ MS. Sesquiterpene compounds are predominant in *Piper borbonense* C. DC. essential oils, but in *Piper capense* L. f. essential oil, monoterpene compounds were predominant. Antioxidant activity was evaluated by DPPH reduction method. *Piper borbonense* leaves and *Piper capense* essential oils were most active than *Piper borbonense* twigs essential oil, but this activity was less than BHT activity. Essential oils were also investigated for their antimicrobial activity on bacteria and fungi. *Piper borbonense* twigs essential oil was most active against all tested bacterial strains. The lower activity was observed in *Piper borbonense* leaves essential oil. For fungicidal testing, the highest activity was observed in essential oil from *Piper borbonense* leaves compared to that from twigs. The lower activity was observed in *Piper capense* essential oil. These oils are demonstrated a strong activity in vitro as potential antioxidant and antimicrobial agents.

Keywords: *Piper borbonense*, *Piper capense*, chemical composition, antioxidant, antifungal, antibacterial.

INTRODUCTION

Nowadays antimicrobials present increasing inefficacies by the increasing strength and a stunning ability of spreading microorganisms. Consumption of foods contaminated with some pathogenic microorganisms represents also a serious health risk to humans. Therefore, herbs and other natural substances as viable alternatives to pharmaceutical antibiotics became an important voice for scientific research. To prevent contamination during the production, sale and distribution, and to extend the shelf life time of raw and processed foods, synthetic additives were commonly used. However, the safety aspects of these chemical preservatives are discussed since they are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity¹. Thus, since some years, we paid a particular attention to plants and herbs naturally derived compounds as a new alternative to prevent the proliferation of microorganism and protect food from oxidation. Plant extracts have been traditionally used in folk medicine as well as additives to extend the shelf life of foods². Most of their properties are due to secondary metabolites such as the volatile constituents. Essential oil has received a great interest for their potential uses as pharmaceutical alternative medicine and natural food preservatives³. Chemically, they derived

from terpenes and their oxygenated compounds, and each component contributes to their biological activities⁴. So, antifungal and antibacterial effects of essential oils on different microorganisms have been reported in several studies⁵⁻¹³.

Piperaceae is one of the most families of flowering plants. It consists of 12 genera comprising about 1400 species from tropical and sub-tropical region in both hemispheres¹⁴. The family of *Piperaceae* contains several aromatic species used in traditional medicine^{15,16}. The most representative genus of this family is the *Piper*, with approximately 1000 species, from which many have been, described earlier¹⁷. Several *Piper* species have a major economic importance since they are used as spices and traditional medicines¹⁸. For valorization and diversification of medicinal plants used in Comoros, we are interested in two piper species growth in this archipelago. Their chemical composition, antioxidant, antibacterial and antifungal activities were investigated.

MATERIALS AND METHODS

Plant material

The choice of different parts reaped for each plant was guiding by a previous ethnopharmacological investigation¹⁹. Leaves and twigs of *Piper borbonense* C.



P. borbonense (Leaves and twigs)



P. capense (leaves and seeds)

DC. were collected in September 2009 from the Hantsongoma forest (950 m altitude, Oichili province, NE of Ngazidja, Comoros). Seeds of *Piper capense* L.f. were collected in January 2010 from Nkurani ya sima forest (South of Ngazidja) and identified in Comoros National Herbarium. Voucher specimens, CIM 33 and CIM 30 respectively for *Piper borbonense* and *Piper capense*, were deposited in the herbarium of botanic department from Faculty of Sciences and Technology, University of Comoros and National Institute of Medicinal and Aromatic Plants, Morocco.

Essential oil extraction

The plant materials were subjected to hydrodistillation for 3h using a modified Clevenger-type apparatus. For each test 100g of dried leaves or seeds can be used. The oils obtained, were separated from water by decantation, and dried over anhydrous sodium sulphate (20% of the total mass essential oil), filtered, stored at 4°C and analysed by GC and GC-MS. The oil yields were expressed in mL/100g dry plant material.

Essential oil analysis

The different samples of essential oils isolated from two *Piper* species were analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). GC analyses were performed on a Hewlett-Packard (HP 6890) gas chromatograph (FID), equipped with a HP-5 capillary column (30 m x 0.25 mm x 0.25 µm). The temperature was programmed from 50°C after 5 min initial hold to 250°C at 4°C/min. Gas chromatography conditions were as follows: N₂ as carrier gas (1.8 mL/min); split mode was used (Flow: 72.1 mL/min, ratio 1:50); temperature of injector and detector was 275°C. The machine was led by a computer system type "HP ChemStation", managing the functioning of the machine and allowing to follow the evolution of chromatographic analyses. Diluted samples (1/50 in hexane) of 1.2 µL were injected manually.

GC/MS analyses were performed on a Hewlett-Packard equipped with a HP-5MS (Crosslinked 5% PHME Siloxane) capillary column (30 m x 0.25 mm i.d, 0.25 µm film thickness) and coupled with a mass spectrometer (HP 5973). The temperature was programmed 50 to 250°C at 2°C/min. The carrier gas was He (1.5 mL/min) and used split mode (Flow: 112 mL/min, ratio 1:74.7). The different

compounds were confirmed by reference to their MS identities (Library of NIST98 Spectra). MS operating parameters were: ionization voltages 70eV, ion source temperature 230°C, scan mass range 35-450 amu.

Oils constituents were identified by their retention indices relatives to n-alkanes (C8-C24) and by comparison of their mass spectral fragmentation patterns with those reported in literature²⁰.

DPPH radical scavenging activity

Antioxidant activity of these essential oils was determined according the method described by Mighri et al.,²¹ using DPPH radical with minor modifications.

All essential oils were dissolved in methanol at 0.5mg/ml and serial dilutions are made to obtain the concentrations of 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/ml. 1 mL of 0.004% of DPPH solution was mixed with 1mL of testing solution of essential oil and the absorbance taken at 514 nm. Positive control, BHT, is also prepared in the same conditions.

Antimicrobial activity

Microorganisms

Seven microbial strains are chosen for their pathogenicity and their ability to contaminate food. Four bacterial strains (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*) and three fungal strains (*Aspergillus niger*, *Penicillium digitatum* and *Penicillium expansum*).

Bacterial strains are lots of ATCC (American Type Culture Collection) and fungal cultures were obtained from the Centre of Forest Research (Morocco). Cultures of each microorganisms species were maintained on potato-dextrose-agar (PDA) for fungi and tryptic soja agar (TSA) for bacteria and stored at less than 4°C.

Antimicrobial assay:

Antimicrobial assays were performed as described previously by Remmal et al.,²² with minor modifications by Farah et al.²³. The Minimal Inhibitory Concentration (MIC) tests of essential oil were determined according to the method reported by Remmal et al.²².

The essential oils are not miscible in water and culture medium. An emulsion from this oil was realized using solution of agar in 0.2 %. The objective of this technique is to obtain a homogeneous distribution of essential oil to

Table 1: Chemical composition of *Piper borbonense* and *Piper capense* essential oils from Comoros.

KI	Compounds	<i>P. capense</i>	<i>P. borbonense</i> (T)	<i>P. borbonense</i> (L)	Method of Identification
921	α -Thujene	0.20	-	0.10	MS, KI
928	α -Pinene	4.00	-	2.31	MS, KI
943	Camphène	0.28	-	-	MS
971	Sabinene	0.48	-	-	MS
986	Myrcene	0.79	-	0.58	MS, KI
1001	δ -2-Carene	11.13	-	6.6	MS, KI
1006	α -Phellandrene	12.82	-	8.28	MS, KI
1019	δ -3-Carene	3.18	-	1.9	KI
1023	<i>p</i> -Cymene	11.01	-	6.71	MS, KI
1032	β -Phellandrene	-	-	0.14	KI
1042	(<i>Z</i>)- β -Ocimene	0.85	-	0.9	MS, KI
1081	Terpinolene	0.76	-	0.33	MS
1094	Linalol	-	-	0.51	MS
1116	trans-Pinene hydrate	0.26	-	-	MS
1193	α -Terpineol	-	-	0.20	MS, KI
1197	cis-Piperitol	0.31	-	0.22	MS, KI
1283	(<i>E</i>)-Anethole	9.51	5.54	1.98	MS, KI
1330	δ -Elemene	-	0.63	-	KI
1350	α -Cubebene	-	-	0.22	MS, KI
1358	Eugenol	0.34	-	0.47	MS, KI
1368	Cyclosativene	0.55	1.14	0.30	KI
1382	α -Copaene	-	0.93	-	KI
1383	Geranyl acetate	4.24	-	2.37	MS, KI
1394	β -Elemene	5.05	-	2.40	MS, KI
1411	α -Gurjunene	1.88	1.09	0.94	MS, KI
1422	β -Caryophyllene	0.75	0.65	0.85	MS, KI
1440	(<i>Z</i>)- β -Farnesene	1.44	-	2.59	MS, KI
1449	α -Himachalene	-	1.04	-	KI
1450	α -Humulene	0.34	9.3	-	MS, KI
1460	Allo-aromadendrene	1.06	-	-	KI
1480	Germacrene D	0.81	4.53	0.38	KI
1495	α -Selinene	-	8.74	-	MS, KI
1497	δ -Selinene	13.95	-	16.27	MS, KI
1501	α -Muurolene	1.01	-	0.88	KI
1506	Germacrene A	1.27	-	0.42	KI
1515	Cubebol	1.19	-	2.01	KI
1521	(<i>E,E</i>)- α -Farnesene	-	24.61	3.05	MS, KI
1520	Myristicin	1.68	-	-	KI
1546	(<i>Z</i>)-Nerolidol	-	3.37	-	KI
1547	Elemol	4.03	-	0.29	KI
1552	Germacrene B	0.46	1.54	1.41	KI
1568	Ledol	-	0.82	-	KI
1571	Spathulenol	-	-	27.72	MS, KI
1580	Caryophyllene oxide	-	-	0.52	MS, KI
1601	Isoamyl nerolate	-	-	0.46	KI
1617	5-epi-7-epi- α -Eudesmol	-	0.36	-	KI
1622	(<i>Z</i>)-Asarone	-	0.94	-	KI
1631	γ -Eudesmol	-	-	0.52	KI
1636	cis-Cadin-4-en-7-ol	-	-	1.56	KI
1640	epi- α -Cadinol	-	-	1.46	MS, KI
1650	β -Eudesmol	-	2.44	0.26	MS, KI
1656	α -Cadinol	-	-	0.14	KI
1684	α -Bisabolol	-	32.32	0.95	MS, KI
	Total	95.63	99.99	99.2	

T: Twigs, L: Leaves, MS: NIST 98 spectra and the literature (Adams 1995), KI: Kováts Indices,

increase at most contact germ / compound. In this solution of agar, dilutions were prepared (1/10th, 1/25th, 1/50th, 1/100th, 1/200th, 1/300th and 1/500th). In test tubes containing each 9 mL of the solid medium PDA (potato-dextrose-agar) for fungi and TSA (tryptic soja agar) for bacteria, sterilized in the autoclave during 20 minutes in 121°C and cooled in 45°C, we added aseptically 1 mL of each of the dilutions so as to obtain the final concentrations (1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000 and 1/5000 v/v). We shook suitably tubes to scatter well the essential oil in the culture medium before pouring them into Petri dishes. The control, containing the culture medium and solution of agar in 0.2 % only, are also prepared. Sowing was done by streaking using a platinum loop calibrated to collect the same volume of inoculums. The incubation is done at 25°C for 7 days for fungi and at 37°C for 48 hours for bacteria. Results are done in triplicate.

RESULTS AND DISCUSSION

Yields and Chemical composition

Two Piper species growing in Comoros islands were used in this study to investigate their chemical composition and antimicrobial activity. Chemical composition of these essential oils is presented in table 1.

The essential oil yields were 3.26%, 0.6% and 3.04% (v/w) respectively for *Piper borbonense* leaves, *Piper borbonense* twigs and *Piper capense* seeds.

In essential oil extracted from the leaves of *Piper borbonense*, 39 compounds were identified representing 99.2% of total constituents. The major components were Spathulenol (27.27%), Bicyclogermacrene (16.27%), α -Phellandrene (8.28%), *p*-Cymene (6.71%), δ -2-Carene (6.6%) and composed 65.58% of the essential oil. Sesquiterpene compounds are the most sub family present in this oil with 67.5% of essential oil constituents. As oil extracted from leaves, essential oil from twigs of *P. borbonense* is also largely constituted by sesquiterpene family with 59.65% of constituents. Eighteen compounds were identified in this essential oil and represent 99.99% of total constituents among which one monoterpene compound ((*E*)-Anethole, 5.54%), one phenylpropanoid (Elemicin, 1.54%), and one ether oxide ((*Z*)-Asarone, 0.94%).

The Major components identified in the essential oil from twigs of *P. borbonense* was α -Bisabolol (32.32%), (*E,E*)- α -Farsenene (24.61%), α -Humulene (9.3%), α -Selinene (8.64%) and composed 74.87% of the essential oil. For *P. capense* essential oil, 31 compounds were identified representing 95.63% of total constituents of essential oil. This oil is largely dominated by Bicyclogermacrene (13.95%), α -Phellandrene (12.82%), δ -2-Carene (11.13%), *p*-Cymene (11.01%), (*E*)-Anethole (9.51%) representing 58.42% of total oil.

As demonstrated in this study, *P. borbonense* essential oils are dominated by sesquiterpenes compounds, 68.3% for *P. borbonense* leaves essential oil and 59.65% for *P. borbonense* twigs essential oil. As reported in literature, *Piper* genera are richness on sesquiterpene compounds²³⁻²⁶. Essential oil from leaves of *P. borbonense* contains spathulenol, an oxygenated sesquiterpene present in

diverse proportions in piper essential oils, as major compound. In our oil, its proportion is lowest than this observed in *P. dilatatum* essential oil from eastern Amazon (40%)²⁷, but highest than this observed in *P. capense* essential oil from Kenya (2.6%)²⁸. Bicyclogermacrene, a hydrocarbon sesquiterpene identified in diverse Piper species, is also identified in our essential oils from leaves of *P. borbonense* and *P. capense*. In this study it represented 16.27% in *P. borbonense* leaves essential oil and 13.95% in *P. capense* essential oil. In *P. capense* essential oil, it is the major component identified. These proportions are higher than their observed in *P. gaudichaudianum* leaves from Riozinho, RS, Brazil²⁹, *P. aduncum* and *P. obliquum* from Eastern Ecuador³⁰, *P. dilatatum* from Paraty, Rio de Janeiro State, Brazil²⁴. These proportions are also higher than this observed in *P. capense* from Kakamega, Kenya²⁸. But in *P. dilatatum* from eastern Amazon, Andrade obtained higher proportion than in our essential oils²⁷. α -Humulene (9.3%), α -Selinene (8.74%) and (*E,E*)- α -Farsenene (24.61%) are also hydrocarbon sesquiterpenes observed in *P. borbonense* twigs essential oil and formed with α -Bisabolol (32.32%) as major components. These hydrocarbons sesquiterpene are also present in other *Piper* species. α -Humulene is present in low percentage in *P. dilatatum*, *P. capense*, *P. sarmentosum* and *P. hostmanianum*^{27,28,31,32}, but it was higher in *P. gaudichaudianum*²⁹. The same observation can be done for α -Selinene which the percentage is high than this observed in *P. guineense*, *P. umbellatum*, *P. gaudichaudianum*, and *P. dilatatum*^{27,32,33}. (*E,E*)- α -Farsenene, the major hydrocarbon sesquiterpene observed in our essential oil, is also observed in several *Piper* species as *P. capense*, *P. guineense*, and *P. umbellatum* from Cameroon³³ and *P. gaudichaudianum*²⁹.

According to the Tchoumboungang study, monoterpene is the abundant compound in the essential oil from seeds of *P. capense*³³. However, compared to his essential oil, our essential oil is dominated by α -Phellandrene (12.82%), δ -2-Carene (11.13%), *p*-Cymene (11.01%), and (*E*)-Anethole (9.51%).

As observed in this study, sesquiterpene compounds are predominated in leaves and twigs, but monoterpene compounds are predominated in seeds. This observation is in accordance with the study of Tchoumboungang et al.³³.

DPPH radical scavenging activity

Antioxidant activity of essential oils of *P. borbonense* and *P. capense* was evaluated *in vitro* using DPPH radical scavenging. Five concentrations were testing for each essential oil. Results are showed in figure 1.

A strong activity was observed in *P. borbonense* leaves and *P. capense* essential oils with respectively 71.43 and 70.33% of reducing ability for the highest concentration. At the same concentration the activity of essential oil from twigs of *P. borbonense* was 53.85%. The near result observed on *P. borbonense* leaves and *P. capense* essential oils for the highest concentration was observed also for IC₅₀, with time the same value of IC₅₀ (30.25 μ g/ml), those of twigs of *P. borbonense* was established at 62.5 μ g/ml. Antioxidant activity of essential oils is generally due to their chemical composition, particularly by the presence of

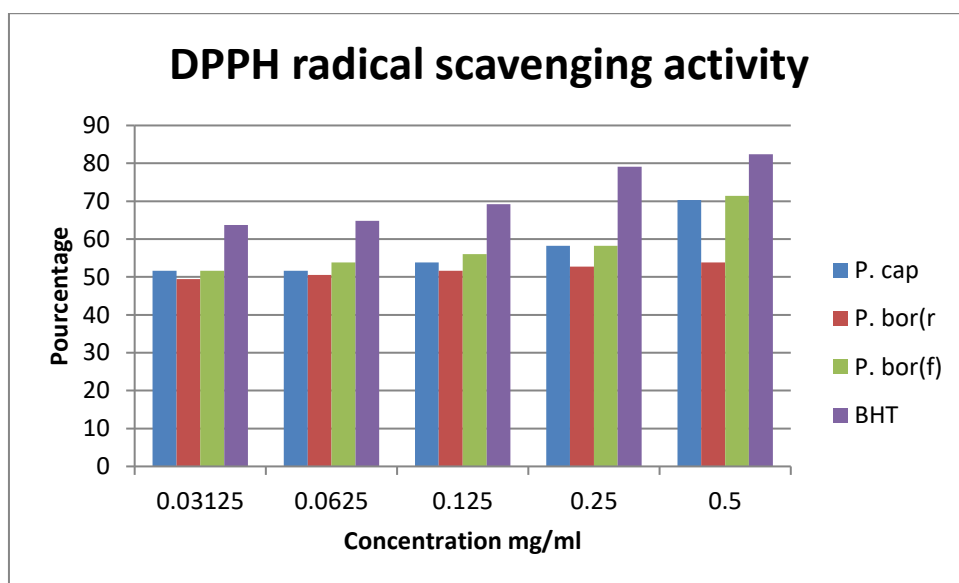


Figure 1: Antioxidant activity of *Piper borbonense* and *Piper capense* essential oils, with P. cap: *Piper capense*, P.bor(f): Leaves of *Piper borbonense*, P.bor(r): twigs *Piper borbonense*

compounds contained a radical function³⁴⁻³⁶. Thus, terpenic alcohols and phenolic compounds are the stronger antioxidant compounds^{37,38}. However our oils are poor in terpenic alcohols and phenolic compounds but have a strong activity, implicated a major role of the other compounds presents in oils. It's also reported that other essential oils poor in phenolic compounds could have stronger antioxidant activity^{39,40}. Indeed by the complexity of their chemical compositions, it's difficult to attribute the antioxidant activity of essential oil to one or another molecule. It's so clear that each molecule, majors and minors, contribute significantly on the essential oil activity^{41,42}.

Antimicrobial activity

Results for antimicrobial activity for essential oils are presented in table 2. The essential oils showed a variable degree of antimicrobial activity against the different strains tested. The gram-positive bacteria, *Bacillus subtilis* were the most sensitive bacteria of the essential oils, followed by the other gram-positive bacteria *Staphylococcus aureus*. While the gram-negative bacterium *Escherichia coli* were the most resistant.

The antibacterial activity showed considerable variations among the different essential oils. The highest activity was manifested by *P. borbonense* twigs essential oil, with inhibitor concentrations of 1/5000 (v/v) for *B. subtilis*, 1/3000 (v/v) for *M. luteus* and *S. aureus* and 1/2000 for *E. coli*.

Similarly, the lowest antibacterial activity was observed in *P. borbonense* leaves essential oil, with inhibitor concentrations of 1/1000 (v/v) for *B. subtilis* and *S. aureus*, and 1/250 for *M. luteus*. This essential oil was inactive against *E. coli*. For *P. capense* essential oil, *B. subtilis* was the most sensitive bacteria. It was inhibited at 1/5000 (v/v). The lowest concentration to inhibit the other bacterium strains was 1/500 (v/v).

For fungicidal activity, the essential oils showed also considerable variations against the different strains. The

highest activity was observed in *P. borbonense* twigs essential oil, with inhibitor concentrations of 1/5000 (v/v) against *Penicillium digitatum* and 1/2000 (v/v) against *Aspergillus niger* and *Penicillium expensum*. *P. borbonense* leaves essential oil was manifested the same activity against all the fungi with an inhibitor concentration of 1/1000 (v/v). The lowest activity was observed in *P. capense* essential oil *A. niger* and *P. expensum* with inhibitor concentration of 1/500 (v/v).

The greatest sensibility of gram-positive bacteria while the gram-negative bacteria showed in this study was also demonstrated in other studies^{5, 43} and can be explained by the presence of double membrane in gram-negative bacteria contrary to gram-positive bacteria that contain only a single membrane².

The difference observed between bacteria and fungi can be explained by the chemical compositions of essential oils. Thus, the higher sensibility of bacteria, observed in this study can explain by the higher percentage of hydrocarbon compounds present in our oils⁴⁴. Similarly, the lowest sensibility on fungi can be also explained by the lowest percentage of oxygenated compounds in our oils⁴⁵.

Biological activity of essential oils may be due to its major components. Therefore the highest activity observed in *P. borbonense* twigs essential oil can be attributed mainly to α -Bisabolol, known for his antimicrobial activities³⁶. However, it is known that by the complexity of the chemical composition of essential, biological activity more pronounced may be due to the presence of synergistical action between the different compounds^{41,42}.

CONCLUSION

Chromatographic analyses are showed a strong dominance of sesquiterpene compounds for *P. borbonense* essential oils and monoterpene compounds for *P. capense* essential oil. For DPPH scavenging activity, *P. borbonense* leaves and *P. capense* essential oils were most active than *P. borbonense* twigs essential oil, but this activity was less

Table 2: Antifungal activity of *Piper borbonense* and *Piper capense* essential oils from Comoros.

V/V		Bacteria				Fungi		
		<i>B. subtilis</i>	<i>E. coli</i>	<i>M. luteus</i>	<i>S. aureus</i>	<i>A. niger</i>	<i>P. digitatum</i>	<i>P. expansum</i>
	<i>P. capense</i>	—	—	—	—	—	—	—
1/100	<i>P. borbonense</i> L	—	+	—	—	—	—	—
	<i>P. borbonense</i> T	—	—	—	—	—	—	—
	<i>P. capense</i>	—	—	—	—	—	—	—
1/250	<i>P. borbonense</i> L	—	+	—	—	—	—	—
	<i>P. borbonense</i> T	—	—	—	—	—	—	—
	<i>P. capense</i>	—	—	—	—	—	—	—
1/500	<i>P. borbonense</i> L	—	+	+	—	—	—	—
	<i>P. borbonense</i> T	—	—	—	—	—	—	—
	<i>P. capense</i>	—	+	+	+	+	—	+
1/1000	<i>P. borbonense</i> L	—	+	+	—	—	—	—
	<i>P. borbonense</i> T	—	—	—	—	—	—	—
	<i>P. capense</i>	—	+	+	+	+	+	+
1/2000	<i>P. borbonense</i> L	+	+	+	+	+	+	+
	<i>P. borbonense</i> T	—	—	—	—	—	—	—
	<i>P. capense</i>	—	+	+	+	+	+	+
1/3000	<i>P. borbonense</i> L	+	+	+	+	+	+	+
	<i>P. borbonense</i> T	—	+	—	+	+	—	+
	<i>P. capense</i>	—	+	+	+	+	+	+
1/5000	<i>P. borbonense</i> L	+	+	+	+	+	+	+
	<i>P. borbonense</i> T	—	+	+	+	+	—	+

(-) : inhibition; (+) : growing, P. ca: *piper capense*, P.bl: Leaves *piper borbonense*, P.bt: Twigs *piper borbonense*, V/V: Concentration of oil

than BHT activity. For antimicrobial activity, *P. borbonense* twigs essential oil was most active on the all bacterial strains tested than *P. capense* essential oil. The less activity was observed in *P. borbonense* leaves essential oil. For fungicidal testing, the highest activity was observed in essential oil from leaves of *P. borbonense* than it is from twigs. The less activity was observed in *P. capense* essential oil.

These oils are demonstrated a strong activity in vitro as potential antioxidant and antimicrobial agents. This is only a preliminary study. Thus, other studies must be conducted with other antioxidant methods and against other microbial strains. We must also determine their mode of action and their possible application in industry like food agro-industry.

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