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Research Article

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

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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 toxicity[1]. 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 foods[2]. 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 preservatives[3]. Chemically, they derived from terpenes and their oxygenated compounds, and each component contributes to their biological activities[4]. So, antifungal and antibacterial effects of essential oils on different microorganisms have been reported in several studies[5-13].

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 hemispheres[14]. 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 earlier[17]. Several Piper species have a major economic importance since they are used as spices and traditional medicines[18]. 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 investigation[19]. Leaves and twigs of *Piper borbonense* C.

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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: N_2 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 literature[20].

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 expensum*).

Bacterial strains are lots of ATCC (Americain Type Culture Collection) and fungal cultures were obtained from the Centre of Forest Research (Morocco). Cultures of each microrganisms 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	*P. capense*	*P. borbonense* (T)	*P. borbonense* (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	*p*-Cymene	11.01	-	6.71	MS, KI
1032	β-Phellandrene	-	-	0.14	KI
1042	(*Z*)-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	(*E*)-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	(*Z*)-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	(*E*,*E*)-α-Farnesene	-	24.61	3.05	MS, KI
1520	Myristicin	1.68	-	-	KI
1546	(*Z*)-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	(*Z*)-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 shaked 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, P*iper* genera are richness on sesquiterpene compounds[23-26]. 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.capanse* essential oil, it is the major component identified. These proportions are higher than their observed in *P.gaudichaudianum* leaves from Riozinho, RS, Brazil[29], *P.aduncum* and *P.obliquum* from Eastern Ecuador[30], *P.dilatatum* from Paraty, Rio de Janeiro State, Brazil[24]. These proportions are also higher than this observed in *P.capense* from Kakamega, Kenya[28]. But in *P.dilatatum* from eastern Amazon, Andrade obtained higher proportion than in our essential oils[27]. 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 Cameroon[33] and *P.gaudichaudianum*[29].

According to the Tchoumbougnang study, monoterpene is the aboundant 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, sesquiterperne compounds are predominated in leaves and twigs, but monoterpene compounds are predominated in seeds. This observation is in accordance with the study of Tchoumbougnang 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 IC_{50}, with time the same value of IC_{50} (30.25µg/ml), those of twigs of *P. borbonense* was established at 62.5 µg/ml.

Antioxidant activity of essential oils is generally due to their chemical composition, particularly by the presence of

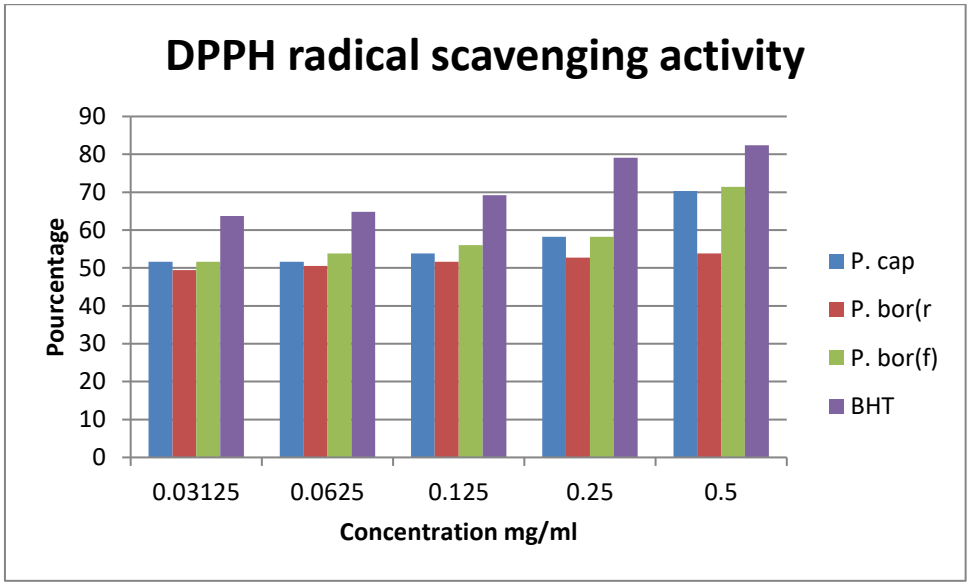

Figure 1: Antioxidant activity of *Piper borbonense* and *Piper capense* essential oils, with P. cap: *Piper capense*, P.bor(f): Leaves of *Piper borbonese*, P.bor(r): twigs *Piper borbonese*

compounds contained a radical function[34-36]. 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 significatively 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 subtilus* 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. subtilus*, 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. subtilus* and *S. aureus*, and 1/250 for *M. luteus*. This essential oil was inactive against *E. coli*. For *P.capense* essential oil, *B. subtilus* 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 membrane[2].

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 oils[44]. Similarly, the lowest sensibility on fungi can be also explained by the lowest percentage of oxygenated compounds in our oils[45].

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 activities[36]. 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		
		B. subtillus	*E. coli*	*M. luteus*	*S. aureus*	*A. niger*	*P. digitatum*	*P. expensum*
	P. capense	—	—	—	—	—	—	—
1/100	*P. borbonese* L	—	+	—	—	—	—	—
	P. borbonese T	—	—	—	—	—	—	—
	P. capense	—	—	—	—	—	—	—
1/250	*P. borbonese* L	—	+	—	—	—	—	—
	P. borbonese T	—	—	—	—	—	—	—
	P. capense	—	—	—	—	—	—	—
1/500	*P. borbonese* L	—	+	+	—	—	—	—
	P. borbonese T	—	—	—	—	—	—	—
	P. capense	—	+	+	+	+	—	+
1/100	*P. borbonese* L	—	+	+	—	—	—	—
0	*P. borbonese* T	—	—	—	—	—	—	—
	P. capense	—	+	+	+	+	+	+
1/200	*P. borbonese* L	+	+	+	+	+	+	+
0	*P. borbonese* T	—	—	—	—	—	—	—
	P. capense	—	+	+	+	+	+	+
1/300	*P. borbonese* L	+	+	+	+	+	+	+
0	*P. borbonese* T	—	+	—	+	+	—	+
	P. capense	—	+	+	+	+	+	+
1/500	*P. borbonese* L	+	+	+	+	+	+	+
0	*P. borbonese* T	—	+	+	+	+	—	+

(-) : inhibition; (+) : growing, P. ca: *piper capense*, $P.b_{L:}$ Leaves *piper borbonese*, $P.b_{T:}$ Twigs *piper borbonese*, 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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REFERENCES

1. Skandamis PN, Nychas GJE. Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. Journal Applied Microbiology 2001; 91(6):1011-1022.
2. Bagamboula CF, Uyttendaele M, Debevere J. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiology 2004; 21(1): 33-42.
3. Alim A, Goze I, Goze HM, Tepe B, Serkedjieva J. In vitro antimicrobial and antiviral activities of the essential oil and various extracts of *Salvia cedronella* Boiss. Journal of Medicinal Plants Research 2009; 3(5): 413–419.
4. Edris AE. Pharmaceutical and therapeutic Potentials of essential oils and their individual volatile constituents: a review. Phytotherapy Research 2007; 21(4): 308-323.
5. Hsouna AB, Trigui M, Mansour RB, Jarraya RM, Damak M, Jaoua S. Chemical composition, cytotoxicity effect and antimicrobial activity of Ceratonia siliqua essential oil with preservative effects against Listeria inoculated in minced beef meat. International Journal of Food Microbiology 2011;148(1): 66–72.
6. Gilles M, Zhao J, An M, Agboola S. Chemical composition and antimicrobial properties of essential oils of three Australian Eucalyptus species. Food Chemistry 2010; 119(2): 731–737.
7. Runyoro D, Ngassapa O, Vagionas K, Aligiannis N, Graikou K, Chinou I. Chemical composition and antimicrobial activity of the essential oils of four *Ocimum* species growing in Tanzania. Food Chemistry 2010; 119(1): 311–316.
8. Kivrak I, Duru ME, Ozturk M, Mercan N, Harmandar M, Topcu G. Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of *Salvia potentillifolia*. Food Chemistry 2009; 116(2): 470–479.
9. Randrianarivelo R, Sarter S, Odoux E, Brat P, Lebrun M, Romestand B, Menut C, Andrianoelisoa HS, Raherimandimby M, Danthu P. Composition and antimicrobial activity of essential oils of Cinnamosma fragrans. Food Chemistry 2009;114(2): 680–684.

10. Chanthaphon S, Chanthachum S, Hongpattarakere T. Antimicrobial activities of essential oils and crude extracts from tropical Citrus spp. against food-related microorganisms. Songklanakarin. Journal of Science and Technology 2008; 30(1): 125-131.
11. Lopes-Lutz D, Alviano DS, Alviano CS, Kolodziejczyk PP. Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. Phytochemistry 2008; 69(8): 1732–1738.
12. Oliveira D R, Leitao G G, Bizzo H R, Lopes D, Alviano DS, Alviano CS, Leitao SG. Chemical and antimicrobial analyses of essential oil of *Lippia origanoides* H.B.K. Food Chemistry 2007; 101(1): 236–240.
13. Burt S. Essential oils: their antibacterial properties and potential applications in foods – a review. International Journal of Food Microbiology 2004; 94(3): 223–253.
14. Sengupta S, Ray AB. The chemistry of *Piper* species: a review. Fitoterapia 1987; 58:147.
15. Constantin MB, Sartorelli P, Limberger R, Henriques AT, Steppe M, Ferreira MJ, Ohara, V.P. Emerenciano MT, Kato MJ. Essential oil from *Piper cernuum* and *Piper regnellii*: antimicrobial activities and analysis by GC/MS and 13C-NMR. Planta Medica. 2001; 67(8): 771-773.
16. Santos PRD, Moreira DL, Guimarães EF, Kaplan MAC. Essential oil analysis of 10 Piperaceae species from the Brazilian Atlantic Forest. Phytochemistry 2001; 58(4): 547–551.
17. Scott IM, Jensen HR, Philogène BJR, Arnason JT. A review of *Piper* spp. (*Piperaceae*) phytochemistry, insecticidal activity and mode of action. Phytochemistry Reviews 2008; 7(1): 65-75.
18. Simpson BB, Ogorzaly MO. Economic Botany: plants in our world. Simpson BB, Ogorzaly MO (eds) 2nd edn. McGraw-Hill Inc., New York, 1995, p 742.
19. Soidrou SH, Ahmed Mohamed N, Farah A, Hassane SOS, Bousta D. Ethnopharmacoligical investigation of five plants used in Comorian folkloric medicine. International Journal of Phytopharmacology 2013; 4(4): 230-236.
20. Adams RP. Identification of essential oil components by gaz chromatography – mass spectroscopy. Carol Steam, In: Allured 1995, p 302.
21. Mighri H, Hajlaoui H, Akrout A, Najjaa H, Neffati M. Antimicrobial and antioxidant activities of Artemisia herba-alba essential oil cultivated in Tunisian arid zone. Comptes Rendus Chimie 2010; 13(3): 380–386.
22. Remmal A, Tantaoui-Elaraki A, Bouchikhi T, Rhayour K, Ettayebi M. Improved method for determination of antimicrobial activity of essential oils in agar medium. Journal of Essential Oil Research 1993 ; 5(2): 179-184.
23. Farah A, Satrani B, Fechtal M, Chaouch A, Talbi M. Composition chimique et activités antibactérienne et antifongique des huiles essentielles d'*Eucalyptus camaldulensis* et son hybride naturel (clone 583). Acta Botanica Gallica 2001 ;148(3): 183-190.
24. Dos Santos PRD, Moreira DL, Guimaraes EF, Kaplan MAC. Essential oil analysis of 10 Piperaceae species from the Brazilian Atlantic forest. Phytochemistry 2001; 58(4): 547- 551.
25. Mundina M, Vila R, Tomi F, Gupta MP, Adzet T, Casanova J, Canigueral S. Leaf essential oil of three panamanian *Piper* species. Phytochemistry 1998; 47(7):1277- 1282.
26. Vila R, Milo B, Tomi F, Casanova J, Ferro E A, Canigueral S. Chemical composition of the essential oil from the leaves of *Piper fulvescens*, a plant traditionally used in Paraguay. Journal of Ethnopharmacology 2001; 76(1):105–107.
27. Andradea EHA, Alves CN, Guimarães EF, Carreira LMM, Maia JGS. Variability in essential oil composition of *Piper dilatatum* L.C. Rich. Biochemical Systematics and Ecology 2011; 39(4-6): 669-675.
28. Matasyoh JC, Wathuta EM, Kariuki ST, Chepkorir R. Chemical composition and larvicidal activity of *Piper capens*e essential oil against the malaria vector, Anopheles gambiae. Journal of Asia-Pacific Entomology 2011; 14(1): 26–28.
29. Péres VF, Moura DJ, Sperotto ARM, Damasceno FC, Caramão EB, Zini CA, Saffi J. Chemical composition and cytotoxic, mutagenic and genotoxic activities of the essential oil from *Piper gaudichaudianum* Kunth leaves. Food and Chemical Toxicology 2009; 47(9): 2389–2395.
30. Guerrini A, Sacchetti G, Rossi D, Paganetto G, Muzzoli M, Andreotti E, Tognolini M, Maldonado M E, Bruni R. Bioactivities of *Piper aduncum* L. and *Piper obliquum* Ruiz & Pavon (Piperaceae) essential oils from Eastern Ecuador. Environnemental Toxicology and Pharmacology 2009 ; 27(1): 39-48.
31. Qin W, Huang S, Li C, Chen S, Peng Z. Biological activity of the essential oil from the leaves of *Piper sarmentosum* Roxb. (Piperaceae) and its chemical constituents on Brontispa longissima (Gestro) (Coleoptera: Hispidae). Pesticide Biochemistry and Physiology 2010; 96(3): 132–139.
32. De Morais S M, Facundo VA, Bertini LM, Cavalcanti ESB, Joao FA Junior, Ferreira SA, Brito ES, Neto MAS. Chemical composition and larvicidal activity of essential oils from *Piper species*. Biochemical Systematic and Ecology 2007; 35(10): 670-675.
33. Tchoumbougnang F, Jazet D P M, Sameza M L, Fombotioh N, Wouatsa N AV, Amvam ZPH, Menut C. Comparative essential oils composition and insecticidal effect of different tissues of *Piper capense* L., *Piper guineense* Schum. et Thonn., *Piper nigrum* L. and *Piper umbellatum* L. grown in Cameroon. African Journal of Biotechnology 2009; 8(3): 424-431.
34. Bouhdid S, Skali SN, Idaomar M, Zhiri A, Baudoux D, Amensour M, Abrini J. Antibacterial and antioxidant activities of *Origanum compactum* essential oil. African Journal of Biotechnology 2008; 7(10): 1563-1570.
35. Chun SS, Vattem DA, Lin YT, Shetty K. Phenolic antioxidants from clonal oregano (*Origanum vulgare*) with antimicrobial activity against *Helicobacter pylori*. Process Biochemistry 2005; 40(2): 809-816.

36. Gülçin I, Sat IG, Beydemir S, Elmastas M, Küfrevioglu OI. Comparison of antioxidant activity of clove (Eugenia caryophylata Thunb) buds and lavender (Lavandula stoechas L.). Food Chemistry 2004; 87(3): 393-400.

37. Fayed SA. Antioxidant and Anticancer Activities of *Citrus reticulate* (Petitgrain Mandarin) and *Pelargonium graveolens* (Geranium) Essential Oils. Research Journal of Agriculture and Biological Sciences 2009; 5(5): 740-747.
38. Zhuang SR, Chen SL, Tsai JH, Huang CC, Wu TC, Liu WS, Tseng HC, Lee HS, Huang MC, Shane GT, Yang CH, Shen YC, Yan YY, Wang CK. Effect of citronellol and the Chinese medical herb complex on cellular immunity of cancer patients receiving chemotherapy/radiotherapy. Phytotherapy Research 2009; 23(6): 785-90.
39. El-Massry KF, El-Ghorab AH, Farouk A. Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. Food Chemistry 2002; 79(3): 331-336.
40. Zouari N, Fakhfakh N, Zouari S, Bougatef A, Neffati M, Ayadi M.A, Karray A. Chemical composition, angiotensin I-converting enzyme inhibitory, antioxidant and antimicrobial activities of essential oil of Tunisian *Thymus algeriensis* Boiss. et Reut. (Lamiaceae). Food and Bioproducts Processing 2011; 89(4): 257–265.
41. Bakkali F, Averbeck S, Idaomar M. Biological effects of essential oils- A review. Food and Chemical Toxicology 2008; 46(2): 446- 475.
42. Wang W, Wu N, Zu YG, Fu YJ. Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. Food Chemistry 2008; 108(3): 1019–1022.
43. Cardile V, Russo A, Formisano C, Rigano D, Senatore F, Arnold NA, Piozzi F. Essential oils of Salvia bracteata and Salvia rubifolia from Lebanon: Chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. Ethnopharmacology 2009; 126(2): 265-272.
44. Yangui T, Bouaziz M, Dhouib A, Sayadi S. Potential use of Tunisian Pituranthos chloranthus essential oil as a natural disinfectant. Letters in Applied Microbiology 2009; 48(1): 112–117.
45. Kordali S, Kotan R, Mavi A, Cakir A, Ala A, Yildirim A. Determination of the chemical composition and antioxidant activity of the essential oil of Artemisia dracunculus and of the antifungal and antibacterial activities of Turkish Artemisia absinthium, A. dracunculus, Artemisia santonicum, and Artemisia spicigera essential oils. Journal of Agricultural and Food Chemistry 2005; 53(24): 9452–9458.