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Chemical composition and biological activities of essential oils of *Pimenta racemosa* (Mill.) J. W. Moore. from Benin

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Abstract

The aim of the present work was to assess potential antiradical, anti-inflammatory and antimicrobial activities of essentials oils of *Pimenta racemosa* from Benin. The chemical compositions of the essential oils obtained by hydrodistillation from fresh leaves of six samples of *Pimenta racemosa* (Mill.) J. W. Moore. (Myrtaceae) growing wild in Benin were analyzed by GC and GC/MS and showed twenty four compounds identified and quantified in the essential oils with eugenol (45.2% - 52.7%), myrcene (25.1% - 29.4%), chavicol (7.1% - 9.3%), limonene (3.0% - 4.0%), 1,8-cineole (2.1% - 3.2%) as major compounds. The evaluation of biological activities of these oils has shown a low anti-inflammatory activity and high antiradical, acaricidal against *Amblyomma variegatum* and antimicrobial activities against both bacteria and fungi. Fractionation of an eugenol rich sample allowed the identification of the bioactive fractions and their contribution to the efficiency of the whole extract. This study suggests that *P. racemosa* essential oils may be useful in the food industry where the antioxidants are used to delay the degradation of fatty substances. Fractionation of an eugenol rich sample allowed the identification of the bioactive fractions and their contribution to the efficiency of the whole extract. This study suggests that *P. racemosa* essential oils may be useful in the food industry where the antioxidants are used to delay the degradation of fatty substances.

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Introduction

Pimenta racemosa (Mill.) J. W. Moore. (syn. *Pimenta acris* Kostel, *Syzygium racemosum* DC), is cultivated in Venezuela, in Puerto Rico, in the Caribbean, especially in Republic Dominicain and in Jamaica for the production of essential oil exploited in industry. The oil is commonly called "bay oil" or "Myrcia oil". This spice is used to aromatize the food (Leung et Foster, 1996). The culinary uses are the same as those of the "bay-tree sauce" (*Laurus nobilis*). In Benin, this specie is also used for various therapeutic properties. A decoction of bark, taken out of infusion, is used against hypertension. The essential oil extracted from the leaves presents the disinfectants and astringent properties; it is moderately toxic by oral way because of its high percentage of phenol, but as a matter of principle it does not give place to allergic reactions at the man (Opdyke, 1973). It is used in the manufacturing of creams, lotions, detergents, or in the shampoos. It is also used in perfumery (Opdyke, 1973), like febrifuge (Ayedoun *et al.*, 1996). It possesses anti-inflammatory and analgesics properties (Duke, 1986; Robineau, 1991). Essential oil intervenes in the patented formula of a capillary cosmetic composition (Orenga, 2003). The most asserted biological properties are the antimicrobial effects which are explained by the high percentage of phenols. The studies are numerous and we will only quote most recent. Aurore *et al.* (1998) showed that essential oil of three varieties of *Pimenta racemosa* possessed antibacterial activity against *Staphylococcus aureus, Enterococcus faecium, Escherichia coli, Pseudomonas aeruginosa* and *Mycobacterium-smegmatis* and antifungal activity against five strains of fungi *Candida albicans, Aspergillus niger, Abisidia corymbifera, Penicillium verrucosum* and *Cladosporium cladosporioides*; the three "chemical" varieties enumerated are the chemotypes with citral/methyl chavicol-methyl eugenol/and chavicol-eugenol). The essential oil of *Pimenta racemosa* presented a strong antibacterial activity on *Escherichia coli* 0157:H7 (Burt et *al.,* 2002). This oil was active on the bacteria Gram (+) and Gram (-) what indicates potentialities for the use of this microbiostatic variety like agent, disinfectant and disinfecting (Saenz *et al.*, 2004). The oil possessed anti-cancer potentiality (Hartwell, 1970), anti-inflammatory drugs activities (Fernandez 2001a, 2001b), a nematicidal activity (Park *et al.,* 2005), insecticidal activity (Noudogbessi *et al.,* 2008).

Exploring common use of *Pimenta racemosa* as food, we investigated on the antiradical, anti-inflammatory, antimicrobial and acaricidal properties of essential oils of *Pimenta racemosa* samples collected in three localities of Benin with the aim to appraise their potential use as natural preservative.

Material and methods

Plants material and isolation of the essential oils

The plant material was collected in two areas of Benin at Godomey (Samples 1, 2, 3) in April, May 2003 and January 2005 and at Kouhounou (Sample 4, 5, 6), in February 2004, December and April 2003. A voucher specimen was deposited in the Herbarium of the University of Abomey-Calavi. Batches of 200 g of fresh leaves were submitted to hydrodistillation for 2h using a Clevenger-type apparatus. After decantation, the oils were dried over anhydrous Na_2SO_4 and stored in sealed vials below 10°C until using.

Chemical analyses of essential oils

Quantitative and qualitative analyses of the essential oils were carried out by gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS). GC/FID analyses were performed using a Varian CP-3380 GC equipped with a DB1 (100% dimethylpolysiloxane) fitted with a fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 µm) and Supelcowax 10 (polyethylene glycol) fused capillary column (30 m x 0.25 mm, film thickness 0.25 µm); temperature program 50 °-200 °C at 5 °C/min, injector temperature 220 °C, detector temperature 250 °C, carrier gas N_2 at a flow rate of 0.5 mL.min^{-1}. Diluted

samples (10/100, v/v, in methylene chloride) of 2.0 µL were injected manually in a split mode (1/100). The percentage compositions were obtained from electronic integration measurements without taking into account relative response factors. The linear retention indices of the components were determined relatively to the retention times of a series of *n*-alkanes (C_9-C_{20}).

GC/MS analyses were performed using a Hewlett Packard apparatus equipped with a HP1 fused silica column (30 m x 0.25 mm, film thickness 0.25 µm) and interfaced with a quadruple detector (Model 5970). Column temperature was programmed from 70 ° to 200 °C at 10°C/min; injector temperature was 220 °C. Helium was used as carrier gas at a flow rat of 0.6 $mL.min^{-1}$, the mass spectrometer was operated at 70 eV. 2.0 µL of diluted samples (10/100, v/v, in methylene chloride) were injected manually in the split mode (1/100).

The identification of individual compounds was based on the comparison of their relative retention times with those of authentic samples on the DB1 column and by matching the linear retention indices and mass spectra of peaks with those obtained from authentic samples and/or the NBS75K.L and NIST98.L libraries and published data (Adams, 2007; Joulain et König, 1998).

Essential oil fractionation

In order to separate the phenolic compounds (acidic fraction) from the other components (neutral fraction), 2 g of the sample of *Pimenta racemosa* leaves from Godomey (sample 1) were submitted to an acido-basic treatment. The essential oil was dissolved in 100 mL of diethyl ether then treated with NaOH 1 M (30 mL x 3). After decantation, the organic layer was washed with distillated deionized water (20 mL x 3), dried over anhydrous Na_2SO_4, filtered and finally concentrated under vacuum using a rotary evaporator to yield F_{N1} (780 mg). Analysis by GC showing the presence of residual phenolic compounds, 100 mg of the mixture was kept for biological evaluation and the same treatment was applied to the residue to get F_{N2} (570 mg) from the organic layer. The aqueous phases obtained from both processes were combined, acidified by HCl 10% until pH = 6 then extracted by diethyl ether (30 mL x 3); after decantation, the organic layer was washed with water (20 mL x 2), dried over anhydrous Na_2SO_4 and filtered. Evaporation of the solvent under vacuum afforded the fraction F_A (1215 mg).

Biological evaluation

Free radical-scavenging activity: DPPH test

Antiradical activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) following the Mellors and Tappel method (Mellors et Tappel, 1996) adapted to essential oil screening (Avlessi *et al.*, 2005). 1,1-diphenyl-picrylhydrazyl [1898-66-4] was purchased from Sigma-Aldrich and the solutions were prepared with analytical grade solvents purchased from standard commercial sources.

DPPH, was dissolved in ethanol to give a 100 µM solution. To 2.0 mL of the ethanolic solution of DPPH were added 100 µL of a methanolic solution of the antioxidant reference eugenol at different concentrations. The essentials oils and the fractions were tested with the same manner. The control, without antioxidant, is represented by the DPPH ethanolic solution containing 100 µL of methanol. The decrease in absorption was measured at 517 nm after 30 min and at 30°C. All the spectrophotometric measures were performed in triplicate with a SAFAS UV mc2 spectrophotometer, equipped with a multicells/multikinetics measure system and with a thermostated cells-case.

The free radical-scavenging activity of each solution was calculated according to the following equation:

$$SC\% = \frac{A\,(blank) - A\,(sample)}{A\,(blank)} \times 100$$

Antiradical activity, defined as the concentration of test material required to cause a 50% decrease of the initial DPPH absorbance, was determined graphically and expressed as SC_{50} ($mg.L^{-1}$).

Anti-inflammatory activity: lipoxygenase test

Soybean lipoxygenase (EC.1.13.11.12) was purchased from Fluka whereas nordihydroguaiaretic acid (NDGA) [500.38.9] and linoleic acid sodium sulfate (822-17-3) were obtained from Sigma Chemical Co; potassium phosphate buffer 0.1 M, pH = 9 was prepared with analytical grade reagent purchased from standard commercial sources. Deionized water was used for the preparation of all solutions.

Lipoxygenase is known to catalyse the oxidation of unsaturated fatty acids containing 1,4-diene structures. The conversion of linoleic acid to 13-hydroperoxy linoleic acid was followed spectrophotometrically by the appearance of a conjugate diene at 234 nm. Nordihydroguaiaretic acid (NDGA), a known inhibitor of soybean lipoxygenase, was used as a reference drug. The experimental conditions were adapted from those previously used and fully described (Alitonou *et al.*, 2010).

Antifungal activity

Preparation of the culture medium

11.5 g agar of yeast extract (Yeast extract AGAR) and 10 g of anhydrous glucose are mixed with 500 mL of distilled water for the preparation of culture medium. After sterilization and addition 5 mL of oxytetracycline (0.1 %), this medium was cast in limp of Petri dish 9 cm in diameter at a rate of 17 mL.

Detection of the moulds

A quantity of vegetable weighed from gardening culture, fresh tomato fruits and banana leaves was diluted in sterile peptone water in order to detect fungi responsible of their deterioration. 30 min after homogenizing each sample, 0.1 mL of the inocula was spread out on the sterilized mould medium (Yeast Extract Glucose Agar: YEGA) and uniformly. The present limp was incubated at 25 °C ± 1 °C five days aware from day light.

Transplantation and mycelial growth

The moulds detected after examination and identification then, are transplanted (subcultured) using a disc of 6 mm in diameter which carries spores from the anamorph mould on the surface of Petri dish containing the former medium YEGA with tested essential oils at different concentrations or no (positive control). The moulds subcultured were incubated at 25 °C ± 1 °C. The mycelial growth was appreciated every day by measuring the average of two perpendicular diameters passing by the middle of the disc, from the first day till the seventh one at, least 7 days (Khallil, 2001).

The antifungal activity was evaluated by the following equation:

$I = (1 - \frac{d}{dc}) \times 100$ (Chang et al., 2000).

With I : index antifungal; d : diameter growth of Petri dish treated out of essential oil; dc: diameter of growth the control (witness) (Petri dish without essential oil).

Test of determination of the fungistatic or fungicidal activity.

With the experimental concentrations where neither no growth, nor germination was observed, we tested the fungistatic or fungicidal activity. This test consists in taking the mycelial disc not germinated at the end of the incubation of the Petri dish and reintroducing it in a new culture medium (former one) nine without natural extract. If the mycelial growth is always inhibited, the fungicidal activity of the natural extract and in the contrary case, it's spoken about the fongiostatic activity.

Antibacterial activity

Essential oil emulsion

2 mL of Mueller Hinton broth added with 0.02 g/L of phenol red were added 40 µL of essential oil, two drops of Tween 80 has been introduced in a hemolyse test tube and homogenized.

Preparation of bacteria suspensions

This preparation was carried out from the three stocks of tested bacteria. A pure colony of each stock was suspended in 5 mL of Mueller Hinton broth. After incubation at 37 °C for two hours, we

obtained 10^6 CFU/mL corresponding to the scale two of McFarland standard.

Determination of Minimal Inhibitory Concentration (MIC)

The method used was reported by Yehouenou et al. 100 µL of bubble Mueller Hinton broth containing 0.02 g/L of phenol red were distributed in all the 96 wells of microplate. 100 µL of essential oil emulsion (initial solution) were added to the well of the first column except that of the second line and successive dilutions of reason 2 were carried out well by well till the 12th one and the remaining aliquot (100 µL) were rejected. 100 µL of Mueller Hinton which not containing phenol red were introduced on the first well of the first columns and successive dilutions of reason 2 were carried out as before. All the wells of the second column received 100 µL of bacteria suspension except the first line which represents the negative control and the second line, the positive control. The microplate one was finally covered with paper parafilm and was incubated at 37 °C during approximately 18 hours.

Acaricidal activity

The harvest of the ticks was carried out as described by Pamo *et al.,* (2003). The ticks are brought back to the laboratory, in Petri dish in plastic perforated by four small air pockets surroundings one mm of diameter (for airing) where they are identified with the binocular magnifying glass thanks to the key of Walker *et al* (2002) selected according to the size (4.2 ± 0.4) mm and of the weight (0.05 ± 0.01) g for their use for the tests. The sensitivity test consists in putting a definite number of adult ticks (10) in different Petri dish with variable essential oil amount (1µL, 2µL, 4µL, 6µL and 8µL). A witness is carried out under the same experimental conditions or Petri dish does not receive any essential oil amount. The dead number of ticks is counted after 6h. The experiment was carried out three times. The death rate is calculated by using the expression of Abbott (1925).

$$Mc = \frac{Mo - Me}{100 - Me} \times 100$$

Mo = mortality recorded in the treated batches (%); Me = mortality recorded at the witnesses (%); Mc = corrected mortality (%).

Statistical analysis

Data from three independent replicate trials were subjected to statistical analysis using Statistica version 6.0. Differences between means were tested using Z-test.

Results and discussion

Chemical composition of Pimenta racemosa essential oils and of their chemical fractions

The yields of the essential oils obtained by hydrodistillation of fresh leaves of *Pimenta racemosa* collected in two locations of Benin are given in Table 1. They range from 0.9 to 2.4%, with the same order of magnitude as those obtained in the previous studies. The chemical compositions of these essential oils and of their fractions are shown in Table 2. Globally, the essential oils were dominated by aromatic structures p-menthane. All of samples were characterized by high percentage of eugenol (45.2% - 52.7%) accompanied by myrcene (21.9% - 30.9%).

We thus finds a chemical composition "traditional" dominated by two phenolic aromatic components resulting from the shikimic way of the acid (eugenol and chavicol) accompanied by a hydrocarbon acyclic monoterpene, the myrcene. The analyzed samples meet completely the standards recommended by AFNOR **f**or the chemical variety of type "clove" with "eugenol-chavicol" (Abaul *et al*., 1995).

We find a similar chemical composition with that obtained for the essential oils of the leaves of the same species collected in Benin for different area (Ayedoun *et al.,* 1996; Noudogbessi *et al.,* 2006; Jirovetz *et al.,* 2007)**.**

Table 1. Yields (w/w percentage) of essential oils obtained from fresh leaves of *Pimenta racemosa* (Mill.) J. W. Moore. from Benin

Samples	Date and place of harvest	Yields (w/w percentage)
1	April 2003 (Godomey)	1.60
2	May 2003 (Godomey)	1.42
3	January 2005 (Godomey)	0.90
4	February 2004 (Kouhounou)	0.99
5	December 2003 (Kouhounou)	1.78
6	April 2003 (Kouhounou)	2.40

The "neutral" fractions (F_{N1}, F_{N2}), obtained after two successive extractions of the phenolic components by basic treatment of an aliquot of sample 1, contains less and less amounts of eugenol and charvicol and consequently higher relative percentages of the non ionizable components. The acidic fraction (F_A) was exclusively constituted by eugenol and chavicol (respectively 86.2 and 13.8%).

Biological activities

Antiradical effect

Significant free radical scavenging activities were observed for the six oil samples; they were compared to those of the commercial antioxidants eugenol and chavicol, which are widely used as a reference.

We obtain comparable reactivities for the various samples, the most active sample being that of sample 1 (SC_{50} = 1.30 ± 0.06 mg.L^{-1}) and least active being that of sample 6 (SC_{50} = 1.88 ± 0.09 mg.L^{-1}). The activity of eugenol (SC_{50} = 1.60 ± 0.08 mg.L^{-1}), the major constituent of these samples is intermediate between those of these two samples (Table 3).

The two only phenolic compounds identified in these essential oil samples are chavicol and eugenol. In table 3, we deferred the relative percentage of these two phenolic compounds and the SC_{50} of each sample tested; the reactivity of these samples varies between 1.30 and 1.88 mg/L. By comparing the essential oil sample (1) of *P. racemosa* to that of eugenol finds that it is 1.3 times factor more active than pure eugenol.

The same experiments were performed with the extracts obtained by fractionation of sample 1 by acido-basic treatment. Their SC_{50} values were given in Table 3 along with those of the essential oil and of its target component, eugenol. F_A, which was composed almost exclusively by the two phenolic components, eugenol (86.2%) and chavicol (13.8%) showed a radical scavenging activity very high to that of the essential oil (SC_{50} = 0.63 mg.L^{-1} against 1.30 mg.L^{-1}). Furthermore, the scavenging efficiency observed for the neutral fractions F_{N1} and F_{N2}, was much higher (SC_{50} = 1.60 mg.L^{-1} and 32 mg.L^{-1} respectively) than that expected on the basis of their phenolic components content (only 23.0% and 4.0% respectively). The high activity of F_{N1} close to that of the essential oil with nevertheless much lower eugenol content (2.0 times factors) is noteworthy. These results suggest a synergic contribution of other constituents of the essential oils to the antiradical activity.

Table 3. Antiradical activity of *Pimenta racemosa* (Mill.) J W. Moore essential oil (sample 1) and of its fractions obtained by acido-basic treatment.

Composed or extracted	SC_{50} (mg/L)
1	1.30 ± 0.06
2	1.40 ± 0.07
3	1.60 ± 0.08
4	1.85 ± 0.09
5	1.71 ± 0.08
6	1.88 ± 0.09
F_{N1}	1.60 ± 0.08
F_A	0.63 ± 0.03
F_{N2}	32.0 ± 1.60
Eugenol	1.60 ± 0.08

Anti-inflammatory effect

The results obtained from the lipoxygenase tests performed on these essential oils were given in table 4.

At the highest essential oil concentration tested (10 ppm), the inhibition percentages did not reach 0 %. The anti-inflammatory activity was none for this plant. The use of this plant as an anti-inflammatory drug should not be recommended in tradition al medicine.

Table 4. *In vitro* inhibition of soybean lipoxygenase by *Pimenta racemosa* (Mill.) J. W. Moore essential oil

Essential oil sample	Concentrations (ppm)	Inhibition percentage (%)
Pimenta racemosa	10	0

Antifungal effect

The antifungal activity of the essential oil of *Pimenta racemosa* (Mill.) J. W. Moore was evaluated. The following results were obtained (Figures. 1 & 2): We noticed a progressive increase in ratio reduction (antifungal capacity) going from (0 to 92.9 %) and (0 to 92.7 %) during the 7 days with the four concentrations (5 µL, 10 µL, 20 µL, 40 µL) of essential oil of *Pimenta racemosa* (Mill.) J. W. Moore tested (Figures 1 & 2) against *Aspergillus ochraceus* and *Penicillium digitatum* respectively. The essential oils of leaves of *Pimenta racemosa* (Mill.) J. W. Moore is most active against *Aspergillus ochraceus* and *Penicillium digitatum*; it showed a total inhibition of the mycelial growth (fungicidal) to a higher concentration ≥ 5 µL. After having reintroduced the mycelial disc of the Petri dish having for concentration 5µL essential oil in a culture medium nine without natural extract, we noted that this essential oil carried on a fungicidal activity against *Aspergillus ochraceus* and *Penicillium digitatum*. This activity is probably due to the presence of the majority compound (eugenol) or has a synergy between the majority compound and the minority compounds.

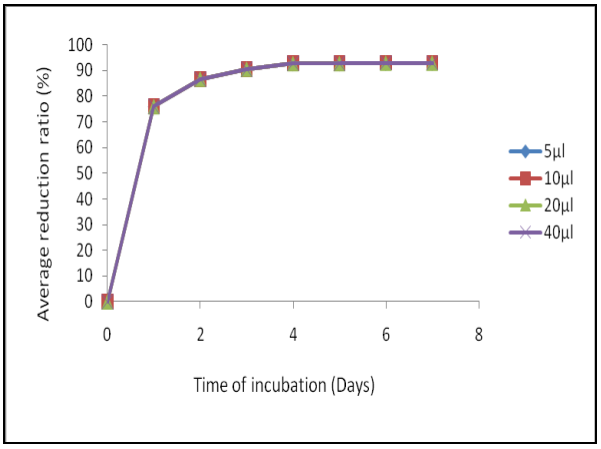

Fig. 1. Action of the oil essential of leaves of Pimenta racemosa (Mill.) J. W. Moore with various concentrations on the mycelial growth of Aspergillus ochraceus.

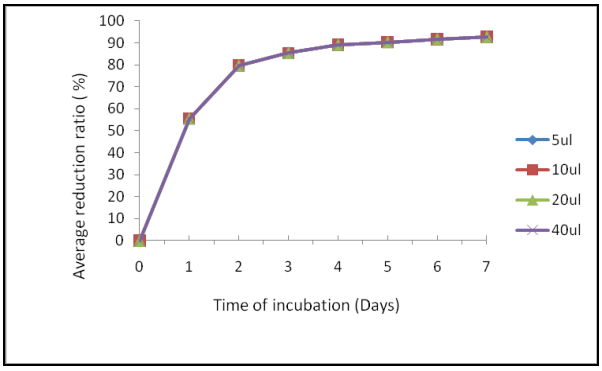

Fig. 2. Action of the oil essential of leaves of Pimenta racemosa (Mill.) J. W. Moore with various concentrations on the mycelial growth of Penicillium digitatum.

Antimicrobial effect

Five microbial stocks were used in this study. The Minimal Inhibitory Concentration (MIC) values were determined for all. The essential oil of the leaves of Pimenta racemosa (Mill.) J. W. Moore almost has an antimicrobial activity very interesting against Candida albicans and Staphylococcus aureus ATCC 25923. MIC = (0.28 ± 0.02) mg/mL and (0.54 ± 0.03) mg/mL respectively, an average activity against the three others microbial stocks (Table 5). These results are similar to those obtained by (Aurore *et al.*, 1998; Burt *et al.*, 2002).

Table 2. Chemical composition of leaf essential oils of *Pimenta racemosa* (Mill.) J W Moore. from Benin and of fractionations obtained by acido-basic treatments of sample 1.

Component	*IR	Sample						F_{N1}	F_{N2}	F_A	Identification methods
		1(%)	2(%)	3(%)	4(%)	5(%)	6(%)	(%)	(%)	(%)	
α-pinene	940	0.4	0.3	0.5	0.6	0.6	0.3	0.3	0.3	-	GC, MS, RI
octen-3-ol	974	2.4	2.1	1.9	1.5	1.4	1.3	6.0	8.1	-	MS, RI
β-pinene	982	0.1	0.1	-	-	-	-	-	-	-	GC, MS, RI
myrcene	993	25.1	25.2	30.9	29.4	29.3	21.9	45.6	53.3	-	GC, MS, RI
α-terpinene	1018	0.1	0.3	0.6	0.6	0.6	0.2	0.2	0.2	-	MS, RI
p-cymene	1022	0.7	0.6	0.4	0.8	0.9	0.8	1.4	1.9	-	GC, MS, RI
limonene	1034	3.0*	3.1*	3.4*	4.0*	3.8*	3.0*	11.9	14.9	-	GC, MS, RI
1,8-cineole		2.7*	2.5*	3.2*	2.4*	2.5*	2.1*	0.3	0.4	-	GC, MS, RI
(E)-β-ocimene	1043	0.2	0.1	0.3	-	0.2	-	-	-	-	GC, MS, RI
γ-terpinene	1058	0.1	0.1	-		0.2	0.1	0.1	-	-	GC, MS, RI
terpinolene	1089	0.2	0.2	-	0.3	-	0.1	0.3	0.2	-	MS, RI
linalol	1092	0.6	0.1	0.3	1.9	2	2.1	1.8	2.6	-	GC, MS, RI
terpinen-4-ol	1178	0.8	0.7	0.7	0.9	0.8	0.8	2.1	3.1	-	GC, MS, RI
α-terpineol	1188	0.7	0.8	0.9	0.8	0.9	0.9	2.4	3.1	-	GC, MS, RI
chavicol	1250	7.5	7.2	8.0	7.1	7.7	9.3	0.1	-	13.8	GC, MS, RI
eugenol	1368	51.1	50.8	46.6	45.5	45.2	52.7	23.0	4.0	86.2	GC, MS, RI
β-caryophyllene	1440	0.1	-	-	0.3	0.3	0.2	0.2	0.3	-	GC, MS, RI
α-humulene	1489	0.1	-	-	-	0.1	0.1	0.1	-	-	GC, MS, RI
(E, E)-α-farnesene	1502	0.2	0.1	0.3	-	0.2	0.2	0.5	-	-	GC, MS, RI
δ-cadinene	1533	0.1	0.1	-	-	0.1	0.2	0.3	-	-	GC, MS, RI
torreyol	1638	0.1	0.1	-	0.3	-	0.2	0.2	0.5	-	MS, RI
t-cadinol	1665	0.1	-	-	-	-	0.2	0.2	0.3	-	MS, RI
diterpene (M+ = 272)	1941	0.6	0.2	-	-	-	0.7	1.2	2.5	-	MS, RI
diterpene (M+ = 272)	1981	0.2	0.1	-	-	-	0.3	0.4	0.9	-	MS, RI
Monoterpene hydrocarbons		29.9	30.0	36.1	35.7	35.6	26.4	59.8	70.8	-	
Oxygenated monoterpenes		4.8	4.1	5.1	6.0	6.2	5.9	6.6	8.8	-	
Sesquiterpene hydrocarbons		0.5	0.2	0.3	0.3	0.7	0.7	1.1	0.3	-	
Oxygenated Sesquiterpene		0.2	0.1	-	0.3	-	0.4	0.4	0.8	-	
Aliphatic derivatives		2.4	2.1	1.9	1.5	1.4	1.3	6.0	8.4	-	
Aromatic composed		58.6	58.0	54.6	52.6	52.9	62.0	23.1	4.0	100	
Total identified		96.4	94.5	98.0	96.4	96.8	97.9	97.0	93.4	100	

- not determined

1 = Collected in April 2003 (Godomey); 2 = Collected in May 2003 (Godomey); 3 = Collected in January 2005 (Godomey); 4 = February 2004 (Kouhounou); 5 = Collected in December 2003 (Kouhounou); 6 = Collected in April 2003 (Kouhounou); F_{N1}, Neutral fraction; F_{N2}, Neutral fraction obtained after basic treatment of F_{N1}; F_A, Acidic fraction;

RI[a], Retention index relative to n-alkanes (C_9-C_{20}) on a DB1 capillary column (100% dimethylpolysiloxane);

Identification methods:

- GC, identification based on retention times of authentic compounds
- MS, identification based on computer matching of the mass spectra of peaks with NBS75K.L, NIST98.L libraries and published data (Adams, 2007; Joulain, König, 1998).

Table 5. Antimicrobial activity (Minimum Inhibitory Concentration: MIC value, mg/mL) of essential oil of leaves of Pimenta racemosa (Mill.) J. W. Moore.

Microbial stocks	Minimum Inhibitory Concentration (MIC) (mg/mL)
Escherichia coli ATCC 25922	2.24 ± 0.13
Staphylococcus aureus ATCC 25923	0.54 ± 0.03
Salmonella typhi	2.24 ± 0.13
Strepto D □*-hemolysute*	2.24 ± 0.13
Candida albicans	0.28 ± 0.02

Antibiotic capacity of the extracts

The averages of the diameters of the halos of incubation measured in mm are consigned in the Table 6 here after:

Table 6. Evaluation of the sensitivity of the microbial stocks tested with respect to antibiotics of reference and of the essential oil of *Pimenta racemosa* (Mill.) J. W. Moore

	E. coli (Diameter mm)				*S. aureus* (Diameter mm)		
EO	Chloramphenicol	Gentamycine	Nalidixique Acide	Ceftriazone	Lyncomycine	Erythromycine	Tetracycline
	18.0 ± 0.7	18.0 ± 0.7	0.0 ± 0.0	21.0 ± 0.8	24.0 ± 0.9	24.0 ± 0.9	30.0 ± 1.2
	30.0 ± 1.2	17.0 ± 0.7	39.0 ± 1.9	0.0 ± 0.0	20.0 ± 0.8	0.0 ± 0.0	30.0 ± 1.2

EO: Essential oil

These results show that *E coli* ATCC 25922 is very sensitive to Chloramphenicol, Gentamycine and Ceftriazone, but resistant to Nalidixic acid. On the other hand the essential oil of *P. racemosa* presents an inhibiting action on the stock compared to Gentamicine in Chloramphenicol and the Nalidixic acid and is without action on the stock compared to Ceftriazone, whereas *S. aureus* is very sensitive to Lyncomycine, Erythromycine and Tetracycline, while the essential oil with an inhibiting activity on *S. aureus* compared to Lyncomycine and with Tetracycline and resists to Erythromycine.

Acaricide effect

The essential oil of *P. racemosa* appeared active with respect to the ticks *Amblyomma variegatum* starting from 2µL. This activity increases as the amount evolves/moves to reach one of mortality of 73.68% with the maximum amount of 8µL (figure 3). This essential oil thus has an insecticidal activity with respect to the ticks. Our results are similar to those of Noudogbessi *et al.*, (2006) which had proven that this oil dominated by eugenol had the insecticidal property on the large capuchin (*Prostephanus truncatus*). This bioactivity could be allotted to the majority compounds of this volatile extract or has a synergy with the minority compounds.

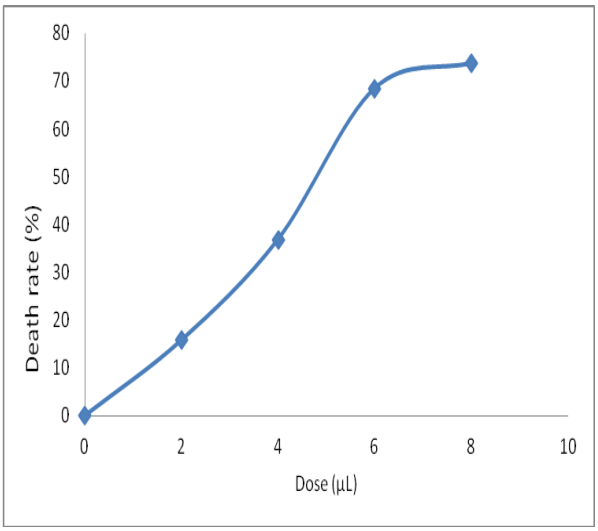

Fig. 3. Evolution of the death rate according to the essential oil amount of *P. racemosa* (Mill.) J. W. Moore

Conclusions

Essential oils compositions of *P. racemosa* from two different locations in Benin were investigated. Eugenol and myrcene rich oils were observed which is similar to the previous literature. The oils showed antimicrobial, antiradical and acaricide activities very interesting. None anti-inflammatory activity was observed. Considering their high content of bioactive constituents, the leaves of *Pimenta racemosa* are exploited in industry and be used in the agroalimentary.

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