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Composition and antimicrobial activity of essential oils of *Cinnamosma fragrans*

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Abstract:

Essential oil samples of Cinnamosma fragrans from two regions in Madagascar, Tsaramandroso (38 samples) and Mariarano (30 samples), were analysed by GC/MS. Fifty-seven components were identified, accounting from 88.3% to 99.4% of the oils' composition. The major components were linalool (72.5 ± 23.3%) in Tsaramandroso and 1,8-cineole (47.3 ± 10.2%) in Mariarano.

Samples B8 (95.8% linalool) from Tsaramandroso and B143 (71.6% 1,8-cineole) from Mariarano containing the highest proportions of the two main components identified, were selected to determine antimicrobial activities against 10 microbial strains. Bacillus subtilis and Staphylococcus aureus were the most sensitive strains to both oils. Minimum inhibitory concentration (MIC) values were lower for B143 against all tested Gram-negative strains than pure 1,8-cineole. B8 showed higher MIC values than pure linalool against Salmonella typhimurium and Vibrio alginolyticus, and similar MIC values to linalool towards the other Gram-negative strains. Both essential oils exhibited higher MIC values towards Fusarium oxysporum than their respective pure major component. These results suggested the occurrence of synergism or antagonism effects between the different oil constituents.

Keywords: *Cinnamosma fragrans*; Essential oil; Antimicrobial activity; Linalool; 1,8-Cineole; Madagascar

1. Introduction

Cinnamosma fragrans Baillon (local names: *Motrobeatiniana*, *Mandravasarotra*), is an endemic tree of Madagascar of the family of *Cannelaceae*. It is distributed extensively in the Northeast of Madagascar {Canonica, 1969 #523}. It grows in the tropophyl forests but often near the coast. Leaves and fruits are very aromatic and have spiced flavour {Perrier de la Bâthie, 1954 #524}. To our knowledge, a few literature describes the chemical composition of this species extracts and the given information is contradictory: Schulte, Rücker and Lewe {, 1972 #526} assumed that linalool is the main compound of essential oil of *C. fragans* isolated from leaves while a recent study {Tucker, 2008 #569} showed that 1,8-cineole and sabinene dominate.

C. fragrans is used traditionally against respiratory, parasitic and gastro-intestinal infections, syphilis {Pernet, 1957 #525; Schulte, 1972 #526}, and malaria {Milijaona, 2003 #527}. Several essential oils components have been reported as efficient antibacterial or antifungal agents, such as linalool {Knobloch, 1989 #520}, 1,8-cineole {Sokmen, 2004 #178}, α-terpineol, terpinen-4-ol, *α*-pinene, β-pinene, β-caryophyllene, α-phellandrene, p-cymene {Dorman, 2000 #489}. *In vitro* studies have demonstrated antibacterial activity of essential oils, *Chaenomeles speciosa* for instance, was active against several pathogens such as *Salmonella sp. Escherichia coli, Bacillus sp., Staphylococcus aureus, Vibrio paraheamolyticus, Fusarium sp,* {Burt, 2004 #177; Xianfei, 2007 #253}.

Vibrio sp. includes many pathogenic or opportunistic strains to aquaculture or to human through the consumption of contaminated seafood {Sung, 1999 #256}. *E. coli, Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus luteus* have been implicated in human diseases {Matasyoh, 2007 #50}. *Fusarium oxysporum* is an abundant and active saprophyte in soil and organic matter, which is a plant pathogenic {Nelson, 1981 #568}.

The aim of the present study was, in a first step, to determine the chemical composition of different samples of the essential oil of *C. fragrans* collected from two sites of the distribution area of the species in Madagascar. In a second step, we examined the *in vitro* antimicrobial activity of two selected samples for their high content in respective linalool and 1,8-cineole against a large selection of Gram-negative (*Salmonella typhimurium, Vibrio spp., Escherichia coli*), Gram-positive bacteria (*Micrococcus luteus, Bacillus subtilis, Staphylococcus aureus*) and one fungus *(Fusarium oxysporum*). We compared those activities with the activity of the major component of each essential oil sample (namely pure linalool and 1,8-cineole).

2. Materials and methods

2.1. Plant material and extraction procedure

For each tree, a sample of leaves (approximately 400 g) was collected during the rainy season, in February 2005: 30 trees were harvested in Mariarano (15° 41'S : 46° 43'E, altitude from sea level to 7 m) and 38 in Tsaramandroso (16° 18' S; 47° 02'E, altitude: 600 m) for a total of 68 samples. A voucher specimen was deposited in the Herbarium of FOFIFA. Antananarivo, Madagascar. The leaves were steam distilled during 4 h in a Clevenger-type apparatus. Distillations were performed less than 24 h after sampling. The essential oils were dried over anhydrous sodium sulphate until the last traces of water were removed and then stored in the dark glass bottle at 4°C.

2.2. Chemical analysis

The 68 samples of essential oils were analysed by gas chromatography (GC) and gas chromatography combined with mass spectrometry (GC/MS).

A Varian 3400 gas chromatograph was used with a flame ionization detector (FID), an on-column injector, a DB-Wax (column A, J&W Scientific, Folsom, CA, USA) fused silica capillary column (60 m x 0.32 mm i.d. x 0.25 μm film). Oven temperature was increased from 50 °C to 200 °C at a rate of 5 °C /min up where it was held for 20 min. Injector and detector temperature was 230 °C. Helium was the carrier gas at 2.0 ml.min^{-1}, dual FID; split ratio, 1:50. Response factors were taken as 1.0 for all compounds, except for limonene (1.3), with reference to *n*-hexanol as internal standard. Linear retention indices were calculated with reference to *n*-alkanes (C_5-C_{22}). Concentrations are given as the average of triplicate analyses.
GS-MC analyses were performed under the same conditions with GC using an AGILENT 5973 gas chromatography equipped with an AGILENT 6890 series mass selective detector. Analytic conditions; injector and transfer line temperatures, 220°C and 240°C, respectively; oven temperature programmed from 50°C to 200°C at 5°C/min; carrier gas, helium at 1ml/min; injection of 0.1µl (10% hexane solution); split ratio, 1:50.
The identifications of the components were based on the comparison of their mass spectra with those of libraries Wiley and NIST (National Institute of Standards and Technology) and literature data {Adams, 2001 #546}, as well as by comparison of their retention indices and co-injections.
Pure linalool and 1,8-cineole were purchased to Aldrich Chimie (Saint Quentin Fallavier, France).

2.3. Antimicrobial activity

2.3.1. Microbial strains

The activity of the essential oils samples was tested towards 10 different microorganisms: Gram negative bacteria were represented by *Escherichia coli* 363, *Vibrio anguillarum*: ATCC 19264, *Vibrio harveyi* ATCC 14126, *Vibrio alginolyticus* ATCC 17749, *Vibrio fisheri* ATCC 49387, *Salmonella typhimurium* ATCC 14028, three Gram positive strains: *Micrococcus luteus* ATCC 10240, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538 and one fungus: *Fusarium oxysporum* ATCC 695.
V. fisheri, *V. harveyi*, *S. aureus* and *B. subtilis* have been purchased from the Collection of Institut Pasteur (Paris, France). The other strains have been gifted by Ifremer (Montpellier. France). These strains were maintained on solid agar using Marine agar (Difco Laboratories, Detroit, USA) for vibrios; Trypticase soy broth (Difco Laboratories, Detroit, USA) for *S. aureus, B. subtilis, E. coli*, *S. typhimurium* and *M. luteus;* and Sabouraud dextrose agar (BBL, Beckton Dickinson Microbiology Systems, USA) for *F. oxysporum.*

2.3.2. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC were determined for samples B8 from Tsaramandroso, B143 from Mariarano and pure linalool and 1,8-cineole as well.
A broth dilution method was used to determine the MIC and the MBC {Destoumieux, 1999 #551}. Stock solutions of essential oils were prepared in sterile distilled water. These suspensions were further diluted from 0.04mg/ml to 23.5 mg/ml in test tubes. 30°C-overnight cultures were inoculated in 900µl of Zobell {Maes, 1992 #543} for *Vibrio* strains or Poor broth {Destoumieux, 1999 #551} for the other strains. The optical density of the inoculum was measured at D_{600} = 0.1 and then diluted to reach a final optical density of D_{600} = 0.001 in the assay. 100 µl of the essential oil dilution were then added to these cultures to reach a final volume of 1 ml. The tests were carried out in triplicate. A positive control containing the bacterial culture without the essential oil and a negative control containing only the medium were performed as well. Tubes were incubated 24h at 25°C for *Vibrio sp*., 30°C for *M. luteus*, 37°C for *E. coli*, *B. subtilis, S. aureus* and *F. oxysporum* (48h of incubation for the fungi).

The MIC was defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. Cell suspensions (0.1 ml) from the tubes showing no growth were subcultured on Zobell and Poor broth agar plates to determine if the inhibition was reversible or permanent. MBC was determined as the highest dilution (lowest concentration) at which no growth occurred on the agar plates {Gachkar, 2007 #141}.

2.4. Statistical analysis

Mean composition of essential oil was given ± SD.
For comparison of MIC and MBC values, tests were made in triplicate. Analysis of variance was performed using ANOVA. Significant differences between means were determined by Fisher's test at the threshold of ($P<0.05$).

3. Results and Discussion

3.1. Chemical composition

The essential oils isolated from the leaves of *C. fragrans* collected in Mariarano and Tsaramandroso were characterized by their high content in 1,8-cineole and linalool respectively (Table 1).
The samples collected in Tsaramandroso afforded essential oils with a large amount of linalool (72.5 ± 23.3%) and its oxides (1.9%), only two other oxygenated monoterpenoids accounting for more than 1% (terpinen-4-ol and 1-terpineol). The monoterpene hydrocarbons, which represent less than 10% of the mixture, are mainly represented by p-menthane structures *α*-phellandrene, *β*-phellandrene*, p*-cymene and terpinolene. Finally, *β*-cubebene, *β*-caryophyllene, germacrene D, δ-cadinene and caryophyllene oxide are the main sesquiterpenoids components. On another hand, in the Mariarano plants, the oxygenated monoterpenes represented also the major chemical class, and they were dominated by 1.8-cineole (47.3 ± 10.2%), the four other main components in this chemical class being 1-terpineol (4.2 ± 3.0%), terpinen-4-ol (2.2 ± 2.0%), geraniol (1.2 ± 2.1%) and linalool (1.1 ± 1.5%). The hydrocarboned monoterpenes are mainly dominated by pinenes, which represent more than 10% of the whole oil. In the group of sesquiterpenes, *α*-copaene (1.4 ± 1.8%), *β*-caryophyllene (1.1 ± 1.8%) and *α*-humulene (0.9 ± 1.9%) are the most abundant.
It appears that essential oils of *C. fragrans* analysed in this work could be therefore classified as "1,8-cineole type" and "linalool type". This result is close to works describing different essential oils in which major components can constitute up to 95% of the essential oil, whereas other components are present only in trace {Bauer, 2001 #536}. For example the chemical compositions of rosemary and sage essential oils were also characterized by the predominant presence of 1,8-cineole, which accounted for 88.9 and 78% of the total oils respectively {Dimitra, 2000 #537}.
As reported in the literature, many factors such as the geographical origin, the genetic factors, the plant material and the season at which the plants were collected may be responsible for the chemical composition of their essential oil {Sivropoulou, 1997 #529}. Andrianoelisoa, Menut, Collas de Chatelperron, Sarraco, Ramanoelina & Danthu {, 2006 #530} have described several chemotypes in *Ravensara aromatica* essential oils. Faleiro et al. {, 2003 #449} have shown two major types for *Thymus* species: one rich in linalool (61.4%) and the second rich both in linalool (44.4%) and 1,8-cineole (37.4%). Chang, Chang, Chang and Cheng {, 2008 #26} have reported variations in the major components of the essential oil extracted from *Cinnamomum osmophloem* leaves among several regions of Taiwan. The major constituent was either trans-cinnamaldehyde at 91.3%, or cinnamyl acetate 56.4%, or camphor 53.7%, or linalool 95.4% according the regions. For *C. fragrans*,

the essential oil composition is very different between the two sites. We can note that these two sites represent the altitudinal limits of the distribution area of *C. fragrans* {Perrier de la Bâthie, 1954 #524}.
However we cannot conclude, on the basis of this only report that the difference of composition of essential oils between Tsaramandroso and Mariarano is of geographical and environmental origin. The hypothesis of a variation with a genetic origin cannot be excluded (as well as that of the interaction between genotype and environment). A more detailed study is under way.

3.2. Antimicrobial activity

The MIC values ranged from 0.18 to 5.88 mg/ml for B8 (95.8 % linalool) and from 0.37 to 11.75 mg/ml for B143 (71.6% 1,8-cineole) samples (Table 2). In the most cases, the MBCs values of B8 and B143 were equivalent to the MICs values (bactericidal effect), except for B8 against *E.coli* and for B143 against *Salmonella, Bacillus* and *Staphylococcus* strains for which the MBCs were higher than the MICs values (bacteriostatic effect). The main components, 1,8-cineole and linalool are well-known for their antibacterial activities {Viljoen, 2003 #550; Knobloch, 1989 #520}. Others minor constituents we found have also been reported for their antimicrobial activity, such as p-cymene, α-pinene, β-pinene, limonene, α-terpinene, α–terpinolene, caryophyllene oxide and camphene {Sökmen, 2004 #178}. Our results demonstrated a higher antibacterial activity against Gram-negative bacteria for linalool compared to 1,8-cineole, which is in agreement with other results {Faleiro, 2003 #449}.
Both B8 and B143 oils exhibited the lowest MICs against two Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*. The method used to assess the antimicrobial activity, and the choice of the test microorganisms vary between publications. In the most literature, Gram-positive organisms appeared to be more sensitive to essential oil than Gram-negative. But others studies did not confirm this observation as Gram-positive bacteria have been found to be less or equally sensitive than Gram-negative bacteria as well {Burt, 2004 #177}. Our results agreed on this observation as two Gram-positive strains were the most sensitive ones whereas *Micrococcus* strain was the most resistant strain to B143 and it was resistant as much as the *Salmonella* strain to B8. The permeability of the bacterial membranes, the presence of porin proteins in Gram-negative bacteria and the intracellular distribution of the oil constituents are key elements that influence the diffusion and the action of the essential oil, hydrophobic components, into the cell. Variation in the essential oil activity is then expected against different group of bacteria. However, further investigations will be required to understand the mechanism of antimicrobial action of essential oil as a mixture of numerous molecules {Lambert, 2001 #547}.
Globally, *C. fragrans* samples exhibited similar MICs against all Gram-positive bacteria than their respective pure major component, linalool and 1,8 cineole. MICs values were lower for B143 against all tested Gram-negative strains than pure 1,8 cineole. B8 showed higher MICs than pure linalool against *Salmonella typhimurium* and *Vibrio alginolyticus*, and similar MICs than linalool towards the others gram-negative strains. Essential oil samples, tested as complex mixtures, may exhibit antimicrobial activities which differ from those of their major component tested solely {Delaquis, 2002 #114}. It has been reported in the literature that the inhibitory activity of an essential oil results from a complex interaction between its different constituents, which may produce, additive, synergistic or antagonistic effects, even for those present at lower concentrations {Xianfei, 2007 #253}. Sibanda et al. {, 2004 #172; #172} who tested the antimicrobial activity of *Heteropyxis dehniae* leaf oil (58.3% linalool as the major component) against different bacteria and fungi, found either higher, lower or similar activities than the pure linalool depending on the tested microorganism. Faleiro et al. {, 2003 #449} have shown that *E. coli* which was susceptible to pure linalool, became highly resistant to the mixture containing linalool plus 1,8-cineole (1:1). Savelev, Okello, Perry, Wilkins and Perry {, 2003 #113} investigated the *in vitro* anticholinesterase activities of eight commercially available terpenoid constituents of *Salvia lavandulifolia* (1,8-cineole, camphor,

α-pinene, β-pinene, borneol, caryophyllene oxide, linalool and bornyl acetate). They found a minor synergy in 1,8-cineole/α-pinene and 1,8-cineole/caryophyllene oxide combinations at higher concentrations, and an antagonism effect in 1,8-cineole/camphor combinations with the interaction index of 2.
Both essential oils exhibited higher MICs towards the fungus *Fusarium oxysporum* than their respective pure major component. Fraternale, Giamperi and Ricci {, 2003 #549} tested the antifungal activity of the oil of *Thymus mastichina* L, which is rich in both 1,8 cineole (55.5%) and linalool (24.5%) against different species of *Fusarium.* The MICs varied in a range from 0.8 mg/ml to 3.2 mg/ml among the 8 tested strains. *F. oxysporum* we tested was not included in those selected strains, but the MICs we found (5.88 mg/ml) was higher. However these results might be in the same range, because our results showed as well higher MICs for pure 1,8 cineole and linalool, due probably to the different testing methods.
In conclusion, our results demonstrated that 1,8-cineole and linalool were the main constituents of essential oil samples of *C. fragrans* originated from two geographical regions in Madagascar. Both samples exhibited a strong antimicrobial activity. Possible synergistic and antagonistic effects may occur in these oil samples against Gram-negative bacteria and *Fusarium oxysporum*. On the basis of these results, essential oil of *C. fragrans* may prove to be a potentially useful antibacterial or antifongic agent.

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Tables

Table 1: Composition of the essential oils of 68 samples (% area) of *Cinnamosma fragrans* harvested in Tsaramandroso (n=30) and Mariarano (n=38).

			Tsaramandroso		Mariarano		Identification
		RI	mean value[d]	B8	mean value[d]	B143	method
	Monoterpenes hydrocarbons						
1	α-pinene	1012	1.0 ± 1.3	0.1	3.5 ± 1.5	1.1	a ; b ; c
2	camphene	1056	1.1 ± 1.6	0.1	4.8 ± 2.2	1.2	a ; b
3	β-pinene	1097	0.9 ± 1.0	0.1	8.0 ± 3.8	2.1	a ; b ; c
4	sabinene	1113	0.9 ± 1.0	0.2	1.8 ± 0.9	3.2	a ; b
5	δ-3-carene	1142	0.5 ± 0.7	0.1	1.7 ± 1.1	0.5	a ; b
6	α-phellandrene	1157	0.2 ± 0.2	tr	0.4 ± 0.7	2.1	a ; b
7	myrcene	1160	0.2 ± 0.4	0.2	0.6 ± 1.0	1.2	a ; b
8	pseudolimonene	1167	0.3 ± 0.4	-	0.7 ± 1.3	0.2	a ; b
9	α-terpinene	1174	1.4 ± 3.7	0.2	0.9 ± 1.5	0.1	a ; b
10	limonene	1191	0.7 ± 2.3	0.2	1.3 ± 1.9	0.6	a ; b
11	β-phellandrene	1201	0.4 ± 0.6	tr	0.4 ± 0.9	0.4	a ; b
12	*(Z)*-β-ocimene	1232	0.7 ± 0.8	-	1.1 ± 1.3	0.2	a ; b
13	*(E)-β*-ocimene	1248	0.2 ± 0.3	-	0.3 ± 0.3	0.1	a ; b
14	*p*-cymene	1263	0.4 ± 1.1	0.2	0.8 ± 1.1	0.5	a ; b
15	terpinolene	1278	0.8 ± 1.4	0.1	0.6 ± 0.8	0.1	a ; b
16	allo-ocimene	1284	0.0 ± 0.0	tr	0.1 ± 0.3	0.1	a ; b
	Total			**1.5**		**13.7**	
	Oxygenated monoterpenes						
17	1,8-cineole	1207	0.5 ± 0.9	0.4	47.3 ± 10.2	71.6	a ; b ; c
18	perillene	1294	tr	0.1	0.1 ± 0.2	tr	a ; b
19	*cis*-linalool oxide (furanoid)	1439	0.7 ± 0.9	0.1	0.5 ± 0.9	0.1	a ; b
20	*trans*-linalool oxide (furanoid)	1468	1.2 ± 2.7	0.2	0.0 ± 0.1	0.1	a ; b
21	citronellal	1472	0.1 ± 0.1	tr	0.2 ± 0.4	0.2	a ; b
22	linalool	1549	72.5 ± 23.3	95.8	1.1 ± 1.5	2.9	a ; b ; c
23	camphor	1505	0.2 ± 0.7	tr	0.2 ± 0.2	0.4	a ; b
24	bornyl acetate	1575	0.2 ± 0.4	-	0.7 ± 0.8	0.3	a ; b
25	myrtenal	1600	0.2 ± 0.4	tr	0.1 ± 0.2	0.2	a ; b
26	terpinen-4-ol	1606	1.5 ± 2.3	0.3	2.2 ± 2.0	2.5	a ; b ; c
27	(*E*)-2,6 dimethyl-3,7-octadien-2,6-diol	1669	0.3 ± 0.6	0.1	0.5 ± 1.3	-	a ; b
28	*α*-terpinyl acetate	1687	0.1 ± 0.2	0.1	0.3 ± 0.5	0.4	a ; b
29	*(Z)*-2,6 dimethyl-3,7-octadien-2,6-diol	1696	0.0 ± 0.1	tr	0.1 ± 0.2	-	a ; b
30	1-terpineol	1700	1.2 ± 1.4	0.2	4.2 ± 3.0	2.1	a ; b ; c
31	neryl acetate	1720	0.0 ± 0.1	tr	0.2 ± 0.5	0.2	a ; b
32	geranial	1735	0.0 ± 0.1	tr	0.2 ± 0.2	0.1	a ; b
33	citronellol	1751	0.0 ± 0.1	-	0.1 ± 0.2	0.1	a ; b
34	nerol	1795	0.8 ± 1.8	-	0.5 ± 0.9	0.1	a ; b
35	isogeraniol	1811	0.1 ± 0.1	-	0.0 ± 0.1	tr	a ; b
36	geraniol	1845	0.8 ± 1.5	tr	1.2 ± 2.1	0.4	a ; b
	Total			**97.5**		**81.1**	
	Sesquiterpene hydrocarbons						
37	*α*-cubebene	1455	0.1 ± 0.2	tr	0.2 ± 0.4	0.2	a ; b
38	*α*-copaene	1480	0.4 ± 1.2	-	1.4 ± 1.8	0.2	a ; b
39	*β*-cubebene	1535	0.9 ± 1.9	tr	0.5 ± 1.0	0.2	a ; b
40	*β* -caryophyllene	1654	0.7 ± 1.3	0.1	1.1 ± 1.8	0.5	a ; b

41	*α*-humulene	1667	0.2 ± 0.3	-	0.9 ± 1.9	0.3	a ; b
42	germacrene-D	1713	0.5 ± 0.7	-	0.7 ± 1.3	0.2	a ; b
43	epi-bicyclosesquiphellandrene	1730	0.0 ± 0.1	tr	0.1 ± 0.2	0.1	a ; b
44	*δ*-cadinene	1760	0.6 ± 1.7	-	0.5 ± 0.9	0.1	a ; b
45	cadina-1,4-diene	1783	0.6 ± 1.4	-	0.0 ± 0.2	0.2	a ; b
46	*cis* calamenene	1853	0.3 ± 1.3	tr	0.4 ± 1.0	0.2	a ; b
	Total			**0.2**		**2.9**	
	Oxygenated sesquiterpenes						
47	caryophyllene oxide	1987	0.4 ± 1.1	0.1	0.4 ± 1.1	0.4	a ; b
48	elemol	2083	0.0 ± 0.1	tr	0.0 ± 0.1	0.1	a ; b
49	nerolidol	2089	0.1 ± 0.1	tr	0.0 ± 0.1	0.1	a ; b
50	γ-eudesmol	2166	0.1 ± 0.1	tr	0.1 ± 0.1	0.2	a ; b
51	torreyol	2147	0.0 ± 0.1	tr	0.0 ± 0.0	0.1	a ; b
52	levomenol	2197	0.0 ± 0.1	0.1	0.1 ± 0.2	0.1	a ; b
53	α -eudesmol	2205	0.0 ± 0.1	tr	0.0 ± 0.1	0.1	a ; b
54	β -eudesmol	2213	0.1 ± 0.2	tr	0.1 ± 0.1	0.4	a ; b
55	driminol	2287	0.0 ± 0.1	-	0.0 ± 0.1	0.1	a ; b
	Total			**0.2**		**1.7**	
	Others						
56	*6*-methyl-5-hepten-2-one	1279	0.4 ± 0.5	0.2	0.3 ± 0.8	tr	a ; b
57	2-hexen-1-ol	1288	0.1 ± 0.4	0.1	0.2 ± 1.0	0.1	a ; b
	Total			**0.3**		**0.1**	

a: Retention Index : DB Wax
b: GC-SM : Library NIST & Wiley
c: Co-injection
[d] Values are mean ± SD of 30 samples of *C. fragrans* essential oils in Tsaramandroso and 38 in Mariarano
tr: trace (between 0.02% and 0.1%), Compounds present in trace amounts (<0.01%) were not registered

Table 2:
Antimicrobial activity of B8 and B143 samples of the essential oils of *Cinnamosma fragrans* from Tsaramandroso (altitude) and Mariarano (littoral) origins respectively and isolated linalool and 1,8-cineole.

	Linalool		1,8-cineole		B8 Tsaramandroso (95.8% linalool)		B143 Mariarano (71.63% 1,8-cineole)	
	MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml
Gram positive								
Micrococcus luteus	5.88a	11.75b	11.75b	11.75b	5.88a	5.88a	11.75b	11.75b
Bacillus subtilis	0.18a	0.18a	0.37b	0.73c	0.18a	0.18a	0.37b	0.73c
Staphylococcus aureus	0.18a	0.18a	0.37b	0.73c	0.18a	0.18a	0.37b	0.73c
Gram negative								
Salmonella tyhimurium	2.93a	5.88c	11.75b	11.75b	5.88c	5.88c	2.93a	5.88c
Escherichia coli	1.47a	1.47a	2.93b	2.93b	1.47a	2.93b	1.47a	1.47a
Vibrio fischeri	0.73a	1.47b	1.47b	1.47b	0.73a	0.73a	0.73a	0.73a
Vibrio anguillarum	1.47a	2.93b	2.93b	2.93b	1.47a	1.47a	1.47a	1.47a
Vibrio harveyi	1.47a	2.93b	2.93b	2.93b	1.47a	1.47a	1.47a	1.47a
Vibrio alginolyticus	1.47a	1.47a	5.88c	5.88c	2.93b	2.93b	1.47a	1.47a
Fungi								
Fusarium oxysporum	2.93a	ND	2.93a	ND	5.88b	ND	5.88b	ND

MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration
Values followed by different letters within a line are significantly different by Fisher's test (p=0.05).
ND : Not determined