Scientia Pharmaceutica (Sci. Pharm.) **70**, 93–99 (2002)

Chemical composition and antibacterial activities of the essential oils of *Plectranthus glandulosus* and *Cinnamomum zeylanicum* from Cameroon

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Two aromatic plants have been selected for chemical investigation on account of their antibacterial activities, *Plectranthus glandulosus* (fresh leaves) and *Cinnamomum zeylanicum* (dried leaves). *P. glandulosus* is used as a medicinal plant, while *Cinnamomum zeylanicum* is used as a common spice in many recipes in Cameroon. The essential oils of the plants have been obtained by hydrodistillation using a Clevenger type apparatus, the yields of oils are about 0.3% and 2.0% respectively.

The essential oil of *P. glandulosus* contains mainly β-thujone (about 31%), p-cymen-8-ol (about 25%) and neral (about 10%) and the essential oil of *C. zeylanicum* is characterised by a high percentage of eugenol (85%).

These essential oils have been tested against three strains of bacteria, namely *Pseudomonas fluorescens*, *Escherichia coli* and *Staphylococcus aureus*; qualitative tests (diffusion through solid medium) and quantitative tests (dilution method) have been carried out. *P. fluorescens* shows a higher resistance to the two oils (MIC values not less than 5000 ppm). *E. coli* is more sensitive to these oils, while against *S. aureus* the essential oil of *C. zeylanicum* still shows a high activity (3500 ppm).

The antibacterial activities of the essential oils could be attributed to the components eugenol as the active component of *C. zeylanicum* and β-thujone and p-cymen-4-ol of the essential oil of *P. glandulosus*.

Keywords: *Plectranthus glandulosus*; *Cinnamomum zeylanicum*; essential oils; antibacterial activity, composition

Introduction

The essential oils from spices or medicinal plants have been known to possess biological activities, notably antimicrobial properties, since ancient times. With the growing interest of the use of essential oils in food and pharmaceutical industries, examination of plant extracts for these properties has become of increasing importance (Baratta et al., 1998; Amvam Zollo et al, 1998; Hughes and Lawson, 1991).

Plectranthus glandulosus (Lamiaceae) and *Cinnamomum zeylanicum* (Lauraceae) leaves are potential sources of essential oils in Cameroon and other tropical areas (Dupriez and De Leener, 1987; Jirovetz et al., 1997 and 1998; Ngassoum et al., 2001,Watt and Breyer-Brandwijk, 1962). *P. glandulosus* is a medicinal plant, widely spread in the savannah area of Cameroon. The plant is used against influenza, coughs and chest complaints (Ngassoum et al.,

2001; Watt and Breyer-Brandwijk, 1962). *C. zeylanicum* has been a traditional spice since ancient times. The leaves or the bark are used in various food applications (Jirovetz et al., 1998).

Therefore, the aim of this work was to analyse the essential oils of the two species, to identify their composition of volatiles, to evaluate the antibacterial activities of the essential oils and to correlate the chemical composition with antibacterial properties.

Results and discussion

The results of GC/FID and GC/MS analysis are given on table 1. The main components (concentration higher than 1.0%; calculated as %-peak area of GC-analysis using a non-polar column) of the essential oil of *Plectranthus glandulosus* fresh leaves are: fenchone (30.8%), terpinolene (25.2%), piperitenone (10.9%), p-cymen-8-ol (3.6%), limonene (3.2%), cis-piperitone oxide (3.0%). The essential oil of dried leaves of *Cinnamomum zeylanicum* comprises eugenol (85.2%) as the main component. Other constituents are (E)-cinnamaldehyde (4.9%), linalool (2.8%) and β-caryophyllene (1.8%).

Table 1: Chemical Composition of essential oils of *C. zeylanicum* and *P. glandulosus* from Cameroon

N°	COMPOUNDS	*Cinnamomum zeylanicum*	*Plectranthus glandulosus*
	Hexane and octane derivatives	0,0	1,4
1	2-Hexenal	tr	-
2	Hexen-3-ol	tr	-
3	Octen-3-ol	tr	-
4	(E)-3-Hexenol	-	1,2
5	(E)-2-Hexenol	-	0,1
6	Hexanol	-	0,1
	Monoterpene hydrocarbons	**3,3**	**36,2**
7	α-Thujene	0,1	tr
8	α-Pinene	0,5	0,6
9	Camphene	0,2	0,3
10	Sabinene	-	tr
11	β-Pinene	0,2	0,3
12	β-Myrcene	0,1	2,2
13	α-Phellandrene	0,9	0,7
14	δ-3-Carene	0,1	1,5
15	α-Terpinene	0,1	0,8
16	p-Cymene	0,4	0,4
17	Dehydro-p-cymene	tr	-
18	Limonene	0,3	3,2
19	cis-ß-Ocimene	-	tr
20	β-Phellandrene	0,1	-
21	trans-ß-Ocimene	0,1	0,6
22	γ-Terpinene	0,1	0,2
23	Terpinolene	0,1	25,2
24	p-Cymenene	-	0,2
	Oxigenated monoterpenes	**93,8**	**58,5**
25	1,8-Cineole	0,3	0,1
26	trans-Sabinene hydrate	-	tr
27	β-thujone	-	30,8
28	Linalool	2,8	tr
29	Linalool oxide	0,1	-

30	Linalyl acetate	tr	-
31	Linalyl propionate	tr	-
32	Menthol	-	tr
34	α-Thujone	tr	-
35	Carvacrol	-	tr
36	Myrtenal	tr	-
37	Neral	-	0,8
38	Nerol	-	tr
39	Perilla aldehyde	tr	-
40	Fenchol	-	1,5
41	cis-p-Menth-2-en-1-ol	-	0,1
42	trans-p-Menth-2-en-1-ol	-	0,5
43	Fenchyl acetate	-	tr
44	Geraniol	tr	-
45	Isopulegone	-	tr
46	Limonene oxide	-	tr
47	Verbenone	-	tr
48	Cuminic alcohol	-	tr
49	Cuminic aldehyde	-	tr
50	Camphor	-	0,7
51	Isoborneol	-	tr
52	Borneol	0,1	0,5
53	Isobornyl acetate	-	tr
54	Norbornyl acetate	-	tr
55	Nootkatone	-	tr
56	Terpinen-4-ol	0,1	0,1
57	3,9-Epoxy-p-mentha-1,8-diene	-	-
58	p-Cymen-8-ol	-	3,6
59	α-Terpineol	0,1	0,3
60	Myrtenol	-	tr
61	Pinocarvone	-	tr
62	Pinocarveol	-	tr
63	cis-Carveol	-	tr
64	Terpinyl acetate	-	tr
65	Piperitone	-	tr
66	cis-Piperitone oxide	-	3,0
67	trans-Piperitone oxide	-	0,5
68	cis-2-Hydroxypiperitone	-	0,1
69	Anethol	-	0,1
70	Thymol	-	0,4
71	trans-2-Hydroxypiperitone	-	0,6
72	4-Hydroxypiperitone	-	0,7
73	Piperitenone	-	1,3
74	Eugenol	85,2	0,1
75	Piperitenone oxide	-	10,9
76	Isopulegone	-	1,8
77	Eugenyl acetate	0,1	-
78	Cinnamaldehyde	4,9	-
79	Cinnamyl acetate	0,1	-
	Sesquiterpene hydrocarbons	**1,8**	**2,1**
80	Ylangene	-	tr
81	α-Copaene	-	0,1
82	β-Elemene	-	0,1
83	δ-Elemene	-	tr
84	γ-Elemene	-	tr
85	Eremophilene	-	tr
86	β-Caryophyllene	1,8	tr
87	cis-α-Bergamotene	-	tr
88	β-Cubebene	tr	-
89	α-Cubebene	tr	-

90	α Farnesene	-	0,2
91	α-Humulene	-	0,1
92	Calarene (β-Gurjunene)	-	0,1
93	γ-Cadinene	tr	-
94	Patchoulene	-	tr
95	δ-Cadinene	-	tr
96	Aromadendrene	-	tr
97	Bisabolene	-	tr
98	Germacrene D	-	1,4
99	α-Muurolene	-	0,1
100	Germacrene-B	-	tr
	Oxygenated sesquiterpenes	**0,5**	**0,4**
101	Bisabolol	-	tr
102	Patchoulenol	-	tr
103	Neryl acetate	tr	-
104	Elemol	tr	-
105	Caryophyllene oxide	0,5	tr
106	Humulene oxide	-	0,1
107	Spathulenol	-	0,1
108	Nerolidol	-	0,2
109	Cedrol	tr	-
110	β-Eudesmol	-	tr
111	γ-Cadinol	tr	-
112	δ-Cadinol	tr	-
113	α-Farnesol	tr	-
	Kaurane derivatives	-	tr
	Fatty acids and their esters	tr	tr
	Hydrocarbons (number higher than 20 C)	-	tr

- = not detected
tr = trace compound (concentration less than 0.1%)

The results of the qualitative and quantitative tests of antibacterial activities are given in tables 2 and 3 respectively: All the bacteria were susceptible with different degrees to the neat oils. The inhibition zones of the neat oil of *C. zeylanicum* is higher for all three strains than those of *P. glandulosus*. Using qualitative tests, the following classification could be made from the most susceptible to the least sensitive strains: *S. aureus* followed by *P. fluorescens* and by *E. coli*. The *C. zeylanicum* oil exerts a higher activity with the minimum value of MIC (2000 ppm) against *E. coli* and the *P. glandulosus* oil shows a lower activity, with the highest MIC value (>10000 ppm) against *P. fluorescens*.

Table 2: Antibacterial activities: Inhibition zone (mm) using the essential oils from Cameroon

Plants ↓ **Bacteria →**	***E. coli***	***P. fluorescens***	***S. aureus***
C. zeylanicum	30	22	20
P. glandulosus	20	12	16
Streptomycine sulfate (2000)	15	12	12

Table 3 : Antibacterial activities : Minimum inhibition concentrations (ppm) of the essential oils from Cameroon

Plants ↓ Bacteria →	***E. coli***	***P. fluorescens***	***S. aureus***
C. zeylanicum	2000	5000	3500
P. glandulosus	3600	>10000	5500

The antibacterial activities of the essential *C. zeylanicum* leaf oil can be attributed especially to the high content of the main compound, eugenol. The activity of *P. glandulosus* oils could be due to oxygenated monoterpenes such as fenchone and piperitenone derivatives as well as monoterpene hydrocarbons such as limonene and terpinolene.

These essential oils with their tested antimicrobial activities will open a new perspective for their use in food and pharmaceutical industries as preservatives or effective molecules in some medicines.

Experimental

Plant material

The fresh leaves of *P. glandulosus* were collected from a natural grown shrub near the University area of Ngaoundere (Cameroon). The dried leaves of *C. zeylanicum* were bought at a local market in Ngaoundere. The species were identified by local botanists and the voucher specimens deposited at the National Herbarium of Yaounde.

Isolation of volatile components

The essential oils were obtained by steam distillation in a Clevenger type apparatus for 4 hours each, with a yield of 0.4% for *P. glandulosus* and 2.0% for *C. zeylanicum* leaves. The oils were dried over anhydrous sodium sulphate and stored at 4°C until analysed.

Bioassay

a.) Microorganisms

A collection of three bacteria including gram positive and negative strains, were used: 1.) gram-positive: *Staphylococcus aureus*, 2.) gram-negative: *Pseudomonas fluorescens* and *Escherichia coli*. All the organisms were maintained at room-temperature on a specific broth, E.M.B. for *E. coli* and nutritive agar for *S. aureus* and *P. fluorescens*.

b.) Preliminary screening

Seeded agar plates were prepared using 20 ml of molten Mueller-Hinton agar and 1 ml of test culture consisting of 10^5 microorganisms. Five discs of Whatman paper n°1 were put on a solidified agar and 10 µl of undiluted or diluted essential oil was layed on. Streptomycin sulfate (2000 ppm) was used as a reference antibiotic. The plates were incubated at 37°C for

24 to 48 hours in the dark. The evaluation of inhibitory activity was carried out by measuring the inhibition zones with a Vernier-caliper.

c.)MIC determination

Minimal inhibitory concentrations (MICs) were determined, as recommended by Institut Pasteur Production (Institute Pasteur, 1980), by the agar dilution method . MICs were defined as the lowest concentrations of each extract that inhibited visible growth on agar. The essential oil was diluted with Tween 80 aqueous solution (1%w/w).

Gas chromatography

A GC-14A with FID and integrator C-R6A-Chromatopac (Shimadzu Co., Japan), and integrator C-R1B-Chromatopac (Shimadzu Co., Japan) were used for GC/FID analyses. Carrier gas: hydrogen; injector-temperature: 220°C; detector-temperature: 280°C; temperature programme: 40°C to 250°C with a rate of 6°C/min.; used columns: 60m x 0.25mm SUPELCOWAX^{R} fused silica (film thickness: 0.25 micron; Supelco, USA) and 30m x 0.32mm bonded FSOT-RSL-200 fused silica (film thickness: 0.25 micron; Biorad Co., Germany). Quantification was carried out by %-peak area calculations (GC/FID using a non-polar column). The identification of single compounds was performed by comparison of the retention-times with reference data (Formacek and Kubeczka, 1982; Davies, 1990; Jennings and Shibamoto, 1980; Kondjoya and Berdaque, 1996; Tudor, 1997; Joulain and König, 1998).

Gas chromatography - Mass spectrometry

For GC/MS analyses a GC-17A with a QP5050 mass spectrometer (Shimadzu Co., Japan) and the data system ProLinea (Compaq Co., USA; software class5k), a GC-17A with a QP5000 mass spectrometer (Shimadzu Co., Japan) and the data system ProLinea (Compaq Co., USA; software class5k), a GC-HP5890 with a HP5970-MSD (Hewlett-Packard Co., USA) and the data system Pentium-PC (Böhm Co., Austria; MSD-ChemStation software) and a GCQ (Finnigan Co., USA) with data sytem Gateway-2000-PS75-PC (Siemens-Nixdorf Co., Germany; GCQ-software) were used. Carrier gas: helium; injector-temperature: 250°C; interface-heating: 280°C; ion source-temperature: 200°C; EI-mode, 70 eV; mass range: 41-550 amu. Temperature programmes and columns see GC/FID part. Mass spectra correlations were carried out with Wiley-, NBS-, NIST- and private aroma library spectra.

Acknowledgements

The authors would like to thank the TWAS (Third World Academy of Sciences), Rome, Italy, for the research grant N° 99-124 RG/CHE/AF/AC, which includes the acquisition of GC/FID instrument for the Department of Applied Chemistry, University of Ngaoundéré, Cameroon.

Literature

Amvam Zollo, P.H., Biyitti, L., Tchoumbounang, F., Menut, C., Lamaty, G., Bouchet, P., 1998. Aromatic Plants of Tropical Central Africa. Part XXXII. Chemical Composition and Antifungal Activity of Thirteen Essential Oils from Aromatic Plants of Cameroon. *Flav. Flagr. J.* 13, 107-114.

Baratta, M.T., Dorman, H.J.D., Deans, S.G., Figueiredo, A.C., Barroso J.G., Ruberto, G., 1998. Antimicrobial

and antioxidant properties of some commercial oils. *Flav.Fragr.J.* 13, 235-244.
Davies, N.W., 1990. Gas Chromatographic Retention Indices of Monoterpenes and Sesquiterpenes on Methyl Silicone and Carbowax 20M Phases. *J.Chromatogr.* 503, 1-24.
Dupriez, H., De Leener, P., 1987. *Jardins et vergers d'Afrique.* Apica, Enda, CTA,Wageningen. Collection Terres et vie. 354p.
Formacek, V., Kubeczka, K.-H., 1982. Essential Oils Analysis by Capillary Gas Chromatography and Carbon-13-NMR Spectroscopy. J. Wiley & Sons, Chichester, 87.
Hughes, B.G., Lawson, L.D., 1991. Antimicrobial action of some essentials oils, *Phytother.Res.* 5, 154-158.
Institut Pasteur, 1980. Lilieux et réactif de laboratoire. Pasteur edition, Paris, pp. 159-166.
Jennings, W., Shibamoto, T., 1980. Qualitative Analysis of Flavour and Fragrance Volatiles by Glass Capillary Gas Chromatography. Academic Press, New York.
Jirovetz, L., Buchbauer, G., Ngassoum, M.B., 1997. GC-MS-analysis of essential oils from Cameroonian plants used as spices in local foodstuff. *Recent Res. Devel. in Agricultural and Food Chem.* 1, 241-255.
Jirovetz, L., Buchbauer, G., Ngassoum M.B., Eberhardt, R., 1998. Analysis and Quality Control of the Essential Oil of the Leaves of *Cinnamomum zeylanicum* L. from Cameroon. *Ernährung/Nutrition*, 22(10), 446-445.
Joulain, D., König, W.A., 1998. The Atlas of Spectral Data of Sesquiterpene Hydrocarbons. E.B. Verlag, Hamburg.
Kondjoya, N., Berdague, J.-L. 1996. A Compilation of Relative Retention Indices for the Analysis of Aromatic Compounds. Edition du Laboratoire Flaveur, Saint Genes Champelle.
Ngassoum, M.B., Jirovetz, L., Buchbauer, G., Fleischhacker, W., 2001. Investigation of Essential Oils of *Plectranthus glandulosus* Hook f. (Lamiaceae) from Cameroon. *J. Essent. Oil Res.* 13, 73-75.
Tudor, E., 1997. Temperature dependence of retention index for perfumery compounds on a SE-30 glass capillary column I. Linear correlation. *J. Chromatogr.* 779, 287-297.
Watt., J.M., Breyer-Brandwijk, M.G. 1962. *The medicinal and the poisonous plants of Southern and Eastern Africa*, 2nd edn., Livingstone, London: p. 524-525.

Received January 25th, 2002
Accepted February 27th, 2002