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Full Length Research Paper

Chemical composition and biological activities of essential oil of *Plectranthus tenuicaulis* (Hook f.) leaves from Gabon

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Essential oils were obtained by hydro distillation of dried leaves from two kinds of *Plectranthus tenuicaulis* (Hook f.) J. k. Morton grown in Gabon and were analyzed using GC-FID, GC-MS, ^{1}H and ^{13}C NMR. The main constituent was *(R), (E)*-6,7-epoxyocimene [*(E)*-myroxide] (72.2 to 83.8%) with small amounts of *(Z)*-6,7-epoxyocimene (0.6 to 1.3%), *(E)* and *(Z)*-β-ocimene (1.1 to 2.4% and 0.6 to 1.7%). The oils were examined for their antioxidant, antimicrobial and antibacterial activities. The antioxidant activity was tested by using an *in vitro* radical scavenging activity test and β-carotene-linoleic acid assays. The essential oils showed antioxidant and DPPH radical scavenging activities, and they displayed the inhibition of lipid peroxidation. The oils were less effective than butylated hydroxytoluene (BHT). The antimicrobial activity of oils was studied by means of agar disc diffusion and broth microdilution methods. The essential oils of *P. tenuicaulis* showed antibacterial activity against microorganisms tested; however, they were unable to inhibit the growth of *Streptococcus pyogenes* and fungal strains. The reference bacteria were the most sensitive to the essential oils. We also observed a significant anticandidal effect.

Key words: *Plectranthus tenuicaulis*, essential oil, GC-MS, antimicrobial activity; antioxidant activity.

INTRODUCTION

*Plectranthus tenuicaulis (*Hook f.) J. K. Morton [syn. *Coleus tenuicaulis* Hook. f; *Plectranthus minimus* (Gürke)], is one of 14 species of the genus *Plectranthus* found in the flora of West Africa (Hutchinston et al., 1963). The plant is a small erect, branched, slender pubescent herb 26 to 36 inches high with dominant purple evergreen.

The aim of this study was to characterize the volatile extract of the species *P. tenuicaulis* which is widely spread in Gabon and traditionally used by local populations. During plant collection, it was noted that an infusion of the leaves is used to treat indigestion, sleep disorders and also by young mothers to promote lactation.

Despite the many papers that have been published on the chemistry of *Plectranthus* (Abdel-Mogib et al., 2002) before our works (Agnaniet et al., 2011; Agrebi et al., 2012), to our knowledge, there are no previous reports on either the biological or chemical activities of the volatile (or non-volatile) compounds of *P. tenuicaulis* (Hook).

In this paper, we report the chemical composition, along

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Abbreviations: AME, Antifungal mixture elios; **BHT,** butylated hydroxytoluene; **DPPH,** 1,1-diphenyl-2-picrylhydrazil; **MBC,** minimum bactericidinal concentration; **MFC,** minimum fungicidal concentration; **MIC,** minimum inhibitory concentration.

with the antimicrobial and antioxidant activities of the essential oil obtained by hydro distillation of dried leaves of *P. tenuicaulis* collected in Gabon.

MATERIALS AND METHODS

P. tenuicaulis leaves were collected in Mopia (South-eastern Gabon) and analyzed using Gas Chomatography (GC-FID), Gas Chromatography-Mass Spectrometry (GC-MS), ^{1}H and ^{13}C NMR (Agnaniet et al., 2011; Agrebi et al., 2012).

Microbial strains

The reference strains used were: *Bacillus cereus* LMG 13569, *Enterococcus faecalis* CIP 103907, *Escherichia coli* CIP 11609, *Listeria innocua* LMG 1135668, *Salmonella enterica* CIP 105150, *Shigella dysenteria* CIP 5451, *Staphylococcus aureus* ATCC 9244, *Proteus mirabolis* 104588 CIP, *S. aureus* ATCC 25293 BHI and *Staphylococcus camorum* LMG 13567 BHI. The clinical strains were *Pseudomonas aeruginosa*, *S. aureus*, *E. faecalis*, *Streptococcus pyogenes* and *Candida albicans* vaginal were isolated in the Laboratory of Medical Biology (Burkina-Faso).

Microbiological methods

The agar disc diffusion method was employed for the screening of antimicrobial activities of the essential oils (NCCLS, 1997). The test was carried out in sterile Petri dishes (90 mm in diameter) containing solid and sterile Mueller-Hinton agar (Becton, Dickinson, USA). The oil absorbed on sterile paper discs (5 µl by Whatman disc of 6 mm diameter), were placed on the surface of the media previously inoculated with microbial suspension 0.1 ml (1 µg per Petri dish). One filter paper disc was placed per Petri dish in order to avoid a possible additive activity, exposed via phase steam, of the components from more than one disc. Each dish was sealed, to prevent evaporation, with a film laboratory and incubated aerobically at 30°C (Gram-negative) or 37°C (Gram-positive) depending on the strain for 24 h, followed by the measurement of the zone diameter of inhibition expressed in mm. Tetracycline (BIO-RAD, Marnes-la coquette, France) and ticarcilline (BIO-RAD, Marnes, la coquette, France) were used as antibiotic reference products. Flucanozole (100 µg) and cetoconazole (100 µg) were used as antifungal reference for the vaginal strain. Antifungal Mixture Elios (AME): Rosemary essential oil, geranium essential oil and lavender essential oil were used as the antifungal compound. All tests were conducted in triplicate.

A broth micro dilution method was used to determine the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) (Agnaniet et al., 2009). All tests were performed in Mueller-Hinton broth (Becton Dickinson, USA). A serial doubling dilution of each essential oil was prepared in 96 wells plates over the range of 0.03 to 8% (v/v). The broth was supplemented by tween 80 (Merck, Germany), at a concentration of 0.1%, to enhance the solubility of the essential oils.

Overnight cultures of each broth strain were prepared in Nutrient Broth (Diagnosis Pasteur, France) and the final concentration in each well was adjusted to 5×10^5 CFU/ml after inoculation. Each inoculum concentration was confirmed by the viable count on Plate Count Agar (Merck, Germany). Positive and negative growth controls were included in each test. The tray was incubated aerobically at 30°C (reference Gram-negative strain) or 37°C (reference and isolated Gram-positive) and MICs were determined. The MICs defined as the lowest concentration of essential oil in which the microorganism tested does not show visible growth. To determine the MBCs, a suspension of 10 µl was taken from each well and inoculated in Mueller-Hinton Agar (Becton Dickinson, USA) during 24 h at 30 or 37°C. The MBCs are defined as the lowest concentration of essential oil (Michel-Briand et al., 1986). Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined for *C. albicans* according to the same protocol.

Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The hydrogen atoms or electron-donating ability of corresponding extracts and butylated hydroxytoluene (BHT) was determined by the laundering of purple-colored DPPH methanol solution. This spectrophotometric assay uses the stable radical DPPH as reagent (Cuendet, 1997; Burits, 2000). Briefly, 0.5 mM DPPH radical solution in methanol was prepared, and then 1 ml of this solution was mixed with 3 ml of the solution of the sample in a final concentration of ethanol. After incubation for 30 min in the dark, the absorbance was measured at 517 nm. Decreasing the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. This activity is given as percentage DPPH radical scavenging, which is calculated with the equation:

% DPPH radical cleaning = [(sample absorbance - control absorbance)] / control absorbance]

DPPH radical scavenging activity was an average of the three repeated measurements.

β-carotene-linoleic acid assay

In this trial, antioxidant capacity was determined by the extent of the inhibition of volatile organic compounds and the formation of the conjugated hydroperoxide diene from linoleic acid oxidation (Dapkevicius, 1998). A solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg of β-carotene dissolved in 1 ml of chloroform (HPLC grade); 25 µl of linoleic acid and 200 mg of tween 80 were added as emulsifier because β-carotene is not soluble in water. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml of distilled water saturated with oxygen was added with vigorous agitation at a rate of 100 ml/min for 30 min; 2500 µl of the reaction mixture was then dispersed in test tubes and different concentrations of essential oil were added. The emulsion system was incubated up to 48 h at room temperature. The same procedure was repeated with a positive control BHT and a blank. After this period of incubation, the absorbance of the mixture was measured at 490 nm. Antioxidant capacity of the extracts was compared with those of the BHT and the blank. Tests were carried out in triplicate.

RESULTS

The hydro distillation of young green clear and light green mature stem purple leaves of *P. tenuicaulis* (*Coleus tenuicaulis*) (Hook) of Gabon produced essential oil with 1.3 and 1.7% (w/w), respectively. The major constituents identified by GC and GC-MS analyses are given in Table 1.

The essential oils were subjected to screening for possible antioxidant activity by two complementary test systems to DPPH free radical scavenging and β-carotene/linoleic acid systems.

Table 1. Percentage composition of *Plecthrantus tenuicaulis* essential oils obtained by hydro-distillation.

RI	Compound	Composition (%)	
		Green leaves	Purple leaves
970	octen-3-ol + sabinene	0.5	0.4
986	myrcene	0.2	0.3
1031	(Z)- β-ocimene	1.1	2.0
1036	(E)- β-ocimene	0.6	0.9
1085	terpinolene	0.4	0.1
1089	6,7-epoxiocimene	0.1	0.4
1090	linalol	0.2	0.4
1094	ipsenol	0.5	0.3
1121	(Z)-6,7-epoxiocimene	1.3	1.3
1141	(E)-6,7-epoxiocimene	83.8	76.0
1160	Rose furane epoxide	0.3	0.2
1172	borneol	0.2	0.2
1182	3-(Z)-hexenyl butanoate	1.3	1.8
1187	M=154	1.0	0.9
1199	(E)-2,6-dimethylocta-3,5,7-trien-2-ol	0.1	0.2
1301	(Z)-2,6-dimethylocta-5,7-dien-2,3-diol	0.5	0.4
1306	(E)-2,6-dimethylocta-5,7-dien-2,3-diol	0.1	0.2
1359	eugenol		0.1
1372	α-copaene	0.1	0.1
1388	β-elemene	0.1	0.2
1432	β-caryophyllene	2.6	4.9
1445	(Z)-β-farnesene	1.1	2.2
1464	α-humulene	0.2	0.4
1485	γ-muurolene		0.1
1490	germacrene D	1.3	2.2
1495	γ-amorphene	0.1	0.4
1497	valencene	0.1	0.1
1518	γ-cadinene		0.1
1523	δ-cadinene		0.2
1530	Selina-3,7-11-diene	0.2	0.1
1604	Caryophyllene oxide	0.1	0.1
1668	intermediol	0.4	0.6
Total		98.5	97.8

BHT was used as positive controls in two test systems. The value of $IC_{[50]}$ essential oil was 18.35 mg/ml. The capacity of essential oil for inhibiting the lipid peroxidation was evaluated by β carotene bleaching test (Figure 1).

Table 2 presents the average inhibition zones. Antimicrobial activity of essential oils from the leaves of *P. tenuicaulis* was first evaluated against 17 strains of bacteria and 4 fungi of different origins by the agar disc diffusion method. Diameter of the inhibition zone of essential oil varies from 8 to 35 mm and 6 to 12 mm respectively for the bacteria and fungi. Other strains were sensitive between 8 to 14 mm: *S. pyogenes*, (14 mm), *E. coli* CIP 11602 (12 mm), and *S. dysenteria* CIP 5451 (11 mm).

The MICs, MFCS and MBCs of *P. tenuicaulis* for tested microorganisms were determined by a broth microdilution method (Table 3). The MIC was equivalent to the MBC, indicating a bactericidal action of oils.

DISCUSSION

The DPPH radical scavenging activity of *P. tenuicaulis* essential oil was high but relatively lower than that of BHT (Figure 2). The capacity of essential oil for inhibiting the lipid peroxidation evaluated by β-carotene bleaching test showed that lipid peroxidation is effectively inhibited by essential oil, RAA value = 94% (30 µg/ml).

The best antioxidant activity of the essential oils was obtained on β carotene/linoleic acid systems. The

Table 2. Antimicrobial activities of *Coleus tenuicaulis* essential oils: Screening by inhibition zone diameters (mm).

Reference strain	Origin	Leaves essential oil	Te[b]	Ti[b]
Bacillus cereus LMG13569	LMG	35	18	50
Enterococcus faecalis CIP103907	CIP	25	19	30
Escherichia coli CIP NCTC11602	CIP	12	22	8
Listeria innocua LMG1135668	LMG	35	14	50
Salmonella enterica CIP105150	CIP	35	16	50
Shigella dysenteria CIP5451	CIP	11	21	31
Staphylococcus aureus ATCC9244	ATCC	30	17	48
Staphylococcus camorum LMG13567	LMG	20	20	16
Proteus mirabilis CIP 104588	CIP	8	15	15
Hospital strain				
Enterococcus faecalis	Foecal	25	20	28
Pseudomonas aeruginosa	Vaginal liquid	11	21	19
Staphylococcus aureus	Vaginal liquid	12	21	27
Streptococcus pyogenes	Vaginal liquid	13	20	24
Fungal strain			Fluc	Gris
Candida albicans ATCC10231	ATCC	10	10	15
Candida albicans ATCC90028	ATCC	12	13	10
Candida albicans (n = 2)	Uro-vaginal liquid	8	13	11

Clinical strains	HE	(E)-ep	AME	HER	HEG	L
Pseudomonas aeruginosa	10	8	8/10	10/15	10	<6
Staphylococcus aureus	10	10	20	15	40	35
Escherichia coli	8	12	11	17	20	16
Aspergillus niger	<6	<6	18/21	<6	60	14/15

a) Tested at a concentration of 5 µl/disc. HE: *Coleus tenuicaulis* essential oils, (E)-ep: (E)-6,7-epoxiocimene; b) Te: Tetracycline , Ti : Ticarcilline; Fluc.: Fluconazole; Gris: Griseofulvine, AME: Antifungal Mixture Elios, HER: Rosemary, HEG: Géranium Bourdon, L : Lavander.

Table 3. Antibacterial (MIC, MBC) and antifungal parameters (MIC, MFC) of *Coleus tenuicaulis* essential oils.

Reference strain	Origin	MIC (%)	MBC (%)
Bacillus cereus LMG13569	LMG	1	1
Enterococcus faecalis CIP103907	CIP	0.5	0.5
Escherichia coli CIP 11602	CIP	>8	ND
Listeria innocua LMG1135668	LMG	1	1
Proteus mirabolis 104588 CIP	CIP	>8	ND
Salmonella enterica CIP105150	CIP	0.5	0.5
Shigella dysenteria CIP5451	CIP	4	8
Staphylococcus aureus ATCC9244	ATCC	0.5	0.5
Staphylococcus camorum LMG13567	LMG	1	1
Hospital strain			
Enterococcus faecalis	Foecal	1	1
Pseudomonas aeruginosa	Vaginal liquid	>8	ND
Staphylococcus aureus	Vaginal liquid	>8	ND
Streptococcus pyogenes	Vaginal liquid	1	1
Fungal strain		**MIC**	**MIF**
Candida albicans ATCC10231	ATCC	>8	ND
Candida albicans ATCC90028	ATCC	8	>8
Candida albicans (n = 2)	Uro-vaginal liquid	>8	ND

ND: not determined**,** values given as percentage.

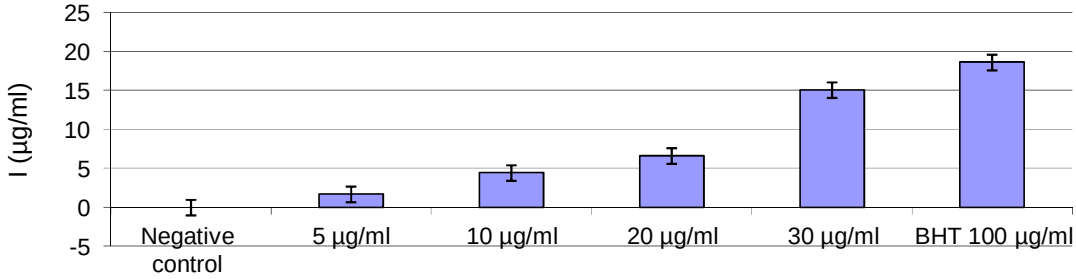

Figure 1. DPPH radical scavenging activity of *Coleus tenuicaulis* essential oils. Y = 13.68 log C-8.3411, R^2 = 0.9018; $IC_{[50]}$ = 18.35 mg/ml. I = %DPPH radical scavenging= [(control absorbance - sample absorbance)]/control absorbance] × 100.

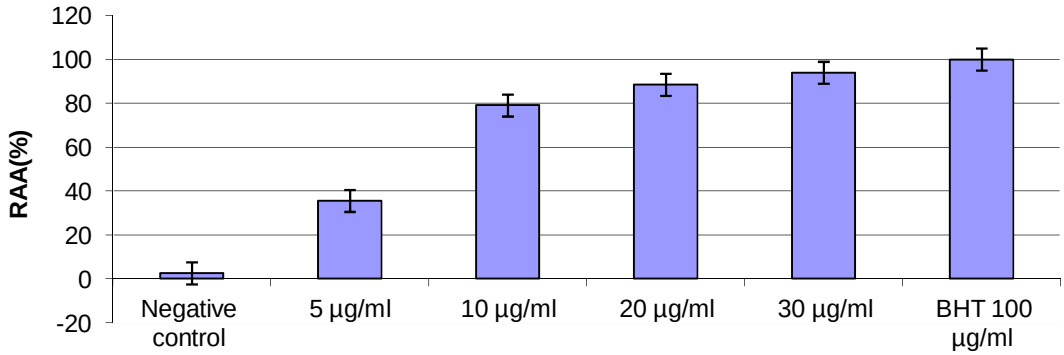

Figure 2. Antioxidant activity by ß-carotene bleaching test of *P. tenuicaulis* essential oils. RAA: Relative Antioxidant Activity = (sample absorbance/BHT absorbance) × 100. BHT: 2,6-di-tert-butyl-4-methylphenol or 2,6-Bis (1,1-dimethylethyl)-4-methylphenol; RAA = 94% (30 µg/ml).

sensitivity to the essential oil was obtained on *P. mirabolis* 104588 PAC (8 mm) and *E. coli* (8 mm) for clinical strains. The different reference strains were less sensitive to the essential oil than tetracycline, and less sensitive to the essential oils than tircacilline.

The essential oils exhibited more activity on *B. cereus* LMG 13569 BHI, *S. enterica* CIP 105150, *E. faecalis* CIP 103907, *E. coli, L. innocua* LMG 1135668 BHI, *S. enterica* CIP 105150, *S. aureus* ATCC 9244 and *S. camorum* LMG 13567 BHI for reference strains and *E. faecalis*, *S. aureus* than tetracycline. The essential oils presented less antifungal activity with an inhibition zone diameter varying from 8 mm for hospital strain candidal, 8 to 12 mm for *C. albicans* ATCC 10231 and *C. albicans* ATCC 90028 and 6 mm for *Aspergillus niger*. The growth of the fungal specie was weak inhibited by *P. tenuicaulis* oil, reference origin *C. albicans* was more sensible to the essential oil than clinical strains of *C. albicans*. It is also interesting to note that the inhibition effect of fluconazole and griseofluvin against *C. albicans* were higher than *P. tenuicaulis* oil. Furthermore, under the experimental conditions, the efficiency was higher for the essential oil than for *(E)*-6,7-epoxyocimene. Two commercial antifungal compounds (fluconazole and griseofluvin), AME, Rosemary essential oil, geranium essential oil and lavender essential oil were more active than the *P. tenuicaulis* essential oil in the case of the clinical strain.

P. tenuicaulis essential oil failed to inhibit *S. enterica* CIP 105150 and *S. aureus* ATCC 9244 (MIC = 0.5%). *P. tenuicaulis* essential oil showed antimicrobial activity (MIC = 1%) on *B. cereus* LMG 13569, *L. innocua* LMG 1135668, *S. camorum* LMG 13567 BHI and *S. pyogenes* (MIC = 1%) for clinical strains.

The result of MBC and MFC demonstrated bactericidal and fungicidal effect. *P. tenuicaulis* essential oil were bactericidal for *B. cereus* LMG 13569 BHI, *S. enterica* CIP 105150, *E. faecalis* CIP 103907, *E. coli* CIP, *L. innocua* LMG 1135668 BHI, *S. enterica* CIP 105150, *S. aureus* ATCC 9244, *S. camorum* LMG 13567 BHI for the reference strains and *E. faecalis*. *P. tenuicaulis* essential oils showed bacteriostatic activity for *S. dysenteria* CIP 5451. The most resistant strains are *E. coli* and *P. aeruginosa*, the latter is known to be resistant to several essential oils (Pattnaik et al., 1995; Agnaniet et al., 2009). This confirms the first screening by the agar diffusion method and is also in agreement with previous reports on the resistance of *P. aeruginosa* (Sonboli, 2005) to several essential oils. Comparison between our data and

anticandidal previously reported data (Leeja et al., 2007) show similar results. Fungal strains were less sensitive to the essential oils of *P. tenuicaulis* leaves.

Conclusion

The present work shows high *in vitro* antimicrobial activity and antioxidant activity of the essential oil of *P. tenuicaulis* leaves. It was bactericidal and low fungicidal for most of the reference and some clinical strains. It is more effective against reference bacteria than clinical bacteria. The essential oils also exhibit an antioxidant activity.

These results indicate that the essential oils from the leaves of *P. tenuicaulis* could be used as a natural antimicrobial agent for infectious diseases and food conservation. Furthermore, the development of natural antimicrobial agents will help to reduce the negative effects (pollution of the environment, resistance), of synthetic chemicals and drugs. The interesting antimicrobial effects support the use of this plant, especially by the local population which has use for good traditional medicine. Nevertheless, it is important to take into account the chemical and biological variability observed, depending on the part of the plant used to make the oil. Further investigation on different sites and periods of harvest are needed to confirm this observation in order to optimize its traditional use.

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