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Full Length Research Paper

Volatile constituents, antioxidant and antibacterial properties of *Daniella Klainei* Pierre ex A. Chev. essential oil

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The water-distilled oil obtained from rhizomes of *Daniella Klainei* Pierre ex A.Chev (Caesalpiniaceae) from Gabon was examined by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The major constituent is myrcene (55.42%) along with α-pinene (5.39%), α-humulène (8.09%) and Germacrene-D (6.06%). The essential oil possessed antioxidant and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities and it displayed the inhibition of lipid peroxidation. A broth micro dilution method was used to test the oil against twelve strains of bacteria; it exhibited an antibacterial activity against some of the microorganisms.

Key words: *Daniella Klainei*, caesalpiniaceae, essential oil, antioxidant, antibacterial.

INTRODUCTION

The genus *Daniella* belongs to the family Caesalpiniaceae. In Gabon, the genus includes five species: *Daniella klainei, Daniella oliveri, Daniella thurifera* and *Daniella ogea*. Among these species, only *D. Klainei* Pierre ex A.Chev has long been used in traditional medicine in Gabon. It is a perennial tree about 45 m height with leaves deciduous to the touch. It is widely distributed in the tropical rain forest (Aubreville, 1970). The resin of this plant is used to heal sores and against microbial infections (our own investigation). The traditional use of this plant suggests antioxidant and antibacterial effects. Recently, there is a growing interest in substances exhibiting antibacterial properties that are supplied to human or animal organisms as specific pharmaceutics. It has been well-known that essential oils have antibacterial activities (Özer et al., 2007). Aromatherapy is now considered to be another alternative way in healing people and the therapeutic values of aromatic plants lie in their volatile components such as terpenoids and phenolic compounds that produce a definite physiological action on the human body (Bruneton, 1987). However, there is so far no report about the antibacterial effects of *D. klainei* essential oil and its chemical composition. On the other hand, the role of free radicals and active oxygen is become increasingly recognized in the pathogenesis of the many human diseases, including cancer, aging and atherosclerosis (Perry et al., 2000). There is no information in literature about the antioxidant activity of any *Daniella* species. Therefore, determination of natural sources of antioxidants and the antioxidant potential of plants is important. This investigation evaluates the antibacterial, antioxidant activities and the chemical composition of the *D. klainei* essential oil.

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MATERIALS AND METHODS

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical was obtained from Fluka; butylhydrazyltoluene (BHT) from Sigma; tetracycline and tircacilline from Bio-Rad Marnes; sodium sulphate and acetone from Prolabo; carotene, linoleic acid and tween 80 from Merck. All the solvents were of analytic grade.

Plant material

D. Klainei Pierre ex A.Chev (Ceasalpiniaceae) resin was collected in March 2007 from Sebang Herbarium of IPHAMETRA, Libreville, Gabon. Voucher specimens are kept in Sebang Herbarium of IPHAMETRA. The essential oil was extracted from resin (500 g) by hydrodistillation in a clavenger-type apparatus for 4 h and was dried, after decantation, over anhydrous sodium sulphate.

Microorganisms

Reference strains used were *Bacillus cereus* LMG 13569, *Enterococcus faecalis* CIP 103907, *Escherichia coli* CIP NCTC 11609, *Listeria innocua* LMG 1135668, *Salmonella enterica* CIP 105150, *Shigella dysenteria* CIP 5451, *Staphylococcus aureus* ATCC 9244, *proteus mirabilis* 104588 CIP, *Staphylococcus aureus* ATCC 25293 BHI, *Staphylococcus camorum* LMG 13567 BHI, *C. albicans* ATCC10231 and *Candida albicans* ATCC90028. While the clinical strains used were *E. faecalis, Pseudomonas aeruginosa, S. aureus and Streptococcus pyogenes.* They were provided by the St Camille Hospital of Ouagadougou, Burkina Faso.

Chromatography

The resin oil was analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). GC analysis was performed on a Hewlett-Packard HP 6890 equipped with a split/splitless injector (280°C), a split ratio 1:10, using a HP-5 capillary column (25 m x 0.25 mm, film thickness 0.25 m). The oven temperature was programmed from 50 to 300°C at a rate of 5°C/mn. Helium was used as the carrier gas at a flow rate of 1.1mL/mn. The injection of each sample consisted of 1.0 L of oil diluted to 10% (v/v) with acetone.

GC/MS analysis is carried out on a Hewlett-Packard 5973/6890 system operating in EI mode (70eV) using two different columns: a fused silica HP-5 MS capillary column (25 m x 0.25 mm, film thickness 0.25 m), and a HP-Innowax capillary column (60 m x 0.25 mm, film thickness 0.25 m). The temperature program for HP-5MS column was 50°C (5 min) rising to 300°C at a rate of 5 °C/min and for the HP-Innowax column, 50-250°C at a rate of 5°C/min. Helium was used as the carrier gas at a flow rate of 1.1 mL/mn. The oil components were identified by comparison of their mass spectra and their retention indices with those of reference compounds or with literature data (Adams, 2001; Joulain and König, 1998; Mc Lafferty and Stauffer, 1989; Van Den Dool and Kratz, 1963).

DPPH radical scavenging activity

The free radical scavenging activity of essential oil was determined according to the method described by Burits and Bucar (2000). Experiments were carried out as described previously (Kordali et al., 2005). 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 sample solution in ethanol. Final concentrations of essential oil were 50, 100 and 150 µg/mL. BHT was used as a positive control at 100 µg/mL concentration. After incubation for 30 mn in the dark, the absorbance was measured at 517 nm. Decrease in the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. This activity is given as percent DPPH radical scavenging, which is calculated with the equation:

$$\text{DPPH radical scavenging (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100.$$

The control contained 1 ml of DPPH solution and 3 ml of ethanol. The measurements of DPPH radical scavenging activity were carried out with three replications, and values are an average of three replicates.

Antioxidant activity

The antioxidant ability of the essential oil was determined according to the method previously described by Dakpevicus et al. (1998). 0.5 mg of β-carotene was dissolved in 1 mL of chloroform (HPLC grade); 25 µL of linoleic acid and 200 mg of tween 40 were added as emulsifier because β-carotene is not water soluble. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 mL of distilled water saturated with oxygen was added with vigorous shaking at a rate of 100 mL/min for 30 min; 2500 µL of this reaction mixture was dispersed to test tubes, and 350 µl portions of extracts, prepared in 2 g/L concentrations, were added. The emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with a positive control BHT and a blank. After this incubation time, the absorbance of the mixture was measured at 490 nm. Antioxidant capacities of the extracts were compared with those at the BHT and the blank. Tests were carried out in triplicate. The relative antioxidant activity (RAA%) of the extracts was calculated from the equation:

$$\text{RAA (\%)} = (A_{sample} / A_{BHT}) \times 100$$

Where A_{BHT} is the absorbance of the positive control BHT and A_{sample} is the absorbance of the extract.

Antibacterial activity essay

A broth microdilution method (Bassole et al., 2003) was used to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). 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 0.03-8% (v/v). The broth was supplemented with Tween 80 at a concentration of 0.1% in order to enhance essential oil solubility. The Tween 80 was to a final concentration of 0.001% (v/v).

Overnight broth cultures of each strain were prepared in Nutrient Broth (Diagnostic Pasteur, France) and the final concentration in each well was adjusted to $5x10^5$ CFU/mL following inoculation. The concentration of each inoculum was confirmed by viable count on Plate Count Agar (Merck, Germany). Positive and negative growth controls were included in every test. The tray was incubated aerobically at 30°C (Gram-negative strains) or 37°C (Gram-positive strains) and MICs were determined. MIC was recorded as lowest concentration of essential oil demonstrating no visible growth in the broth. To determine MBC values, 10 µL of bacterial suspension were removed from each well from MIC essay and inoculated in Mueller-Hinton Agar for 24 h at 30 or 37°C. MBC was defined as a lowest concentration of essential oil killing 99.9% of bacterial inocula (Michel-Briand, 1986). In order to elucidate the antibacterial effect, MBC/MIC ratios were calculated. When the ratio value was lower than 1, essential oil exhibited a bactericidal effect. All tests

Table 1. Constituents of the essential oil of *Daniella klainei* Pierre ex A.Chev.

Peak	RI	Components	%
1	931	α- thujene	0.17
2	939	α- pinene	5.39
3	954	camphene	0.08
4	975	Sabinene	0,50
5	979	β-pinene	2.58
6	991	myrcene	55.42
7	1029	Limonene	0.83
8	1030	β-phellandrene	1.12
9	1033	1,8-cineole	0,40
10	1097	linalool	4.93
11	1177	terpinen-4-ol	0.22
12	1195	α-terpineol	0.27
13	1339	δ- elemene	0.07
14	1351	α-cubebene	0.01
15	1376	α-copaene	1.27
16	1384	β-bourbonene	0.22
17	1390	β-cubebene	0.19
18	1418	β-caryophyllene	3.62
19	1454	α-humulene	8.09
20	1480	germacrene-D	6.06
21	1520	δ-cadinene	0.19
22	1549	Elemol	0.47
23	1561	germacrene-B	0.34
24	1583	Oxyde de caryophyllene	1.02
25	1606	humulene-1,2 epoxyde	1.38
26	1649	β-eudesmol	0.50

RI: retention indices.

were performed in triplicate.

Statistical analysis

Data were expressed as mean ± SEM. A one way variance was use to analyse data. $P<0.01$ represented significant difference between means (Duncan's multiple range test).

RESULTS AN DISCUSSION

Chemical analysis

The hydrodistillation of the resin of *D. klainei* produced essential oil with 3.85% (w/w) yield. The compounds identified in the oil are presented in Table 1 according to their order of elution on HP5. A total of 26 components were identified (95.34%). Myrcene (55.42%), α-pinene (5.39%), α-humulène (8.09%) and Germacrene-D (6.06%) were the main constituents. Monoterpenoids are predominant (71.91%) with oxygenated compounds accounting for 5.89%.

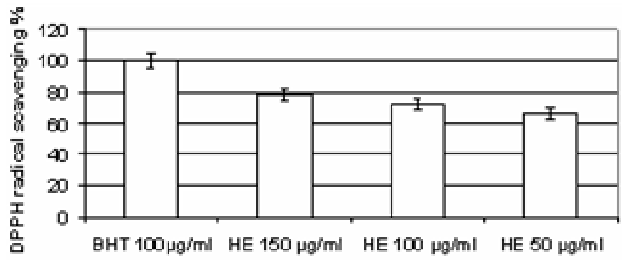

Figure 1. DPPH radical scavenging activity of *Daniella klainei* Pierre ex A.Chev. essential oil.

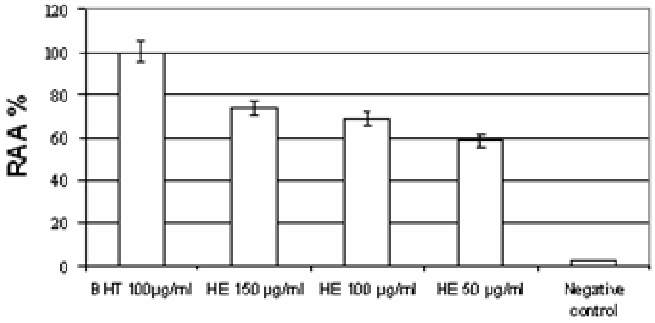

Figure 2. Antioxidant activity by-β-carotene bleaching test of *Daniella klainei* Pierre ex A.Chev. essential oil. RAA = Relative antioxidant activity.

Antioxidant and DPPH radical scavenging activities

The result of DPPH free radicals scavenging activity is reported in Figure 1. The essential oil exhibited a strong scavenging action at 100 µg/mL. However, it showed a weak scavenging activity in comparison to BHT at the same concentration. In the case of the linoleic acid system, the essential oil possessed strong antioxidant capacity for preventing the linoleic acid oxidation, but this effect was lower than that of BHT at 100 µg/mL concentration (Figure 2). The strong antioxidant and DPPH radical scavenging activities of *D. klainei* essential oil can be attributed to the presence of some components that have antioxidant activity; 1,8 cineol, α-pinene, β-pinene (Houghton, 2004) and terpinen-4-ol (Lee and Shibamoto, 2001).

Antibacterial activity

MICs and MBCs varied from 0.50 to 8% for all bacterial strains tested (Table 2). The essential oil was bactericidal for *E. coli* CIP NCTC11602, *S. aureus* ATCC9244, *S. camorum* LMG13567 and *S. aureus*. The most resistant strains with high MIC and MBC were *P. aeruginosa* and *S. pyogenes.* This antibacterial action might be due to the different constituents of the essential oil of this plant such as: 1,8-cineole (Sonboli et al., 2005), terpinen-4-ol and α-

Table 2. Minimum inhibitory concentration and minimum bactericidal concentration of the essential oil of *Daniella klainei* Pierre ex A.Chev (%v/v) obtained by microdilution method.

Strain	Origin	MIC (%)	MBC (%)
Bacillus cereus LMG13569	LMG	1	1
Enterococcus faecalis CIP103907	CIP	2	2
Escherichia coli CIP NCTC11602	CIP	0.5	0.
Listeria innocua LMG1135668	LMG	2	2
Proteus mirabolis CIP 104588	CIP	4	4
Salmonella enterica CIP105150	CIP	1	1
Shigella dysenteria CIP5451	CIP	1	1
Staphylococcus aureus ATCC9244	ATCC	0.5	0.5
Staphylococcus camorum LMG13567	LMG	0.5	0.5
Hospital strains			
Enterococcus faecalis	Foecal	2	4
Pseudomonas aeruginosa	Vaginal liquid	8	8
Staphylococcus aureus	Vaginal liquid	0.5	0.5

Each value represents mean of three different observations.

terpineol (Carson et al., 2006). Almost all the proportions of these components were relatively low in this oil; possible synergistic and antagonistic effects of compounds in the oil should be taken into consideration.

Conclusion

To conclude, our results showed that the essential oil of *D. klainea* is characterized by a high proportion of myrcene (55.42%) along with α-pinene (5.39%), α-humulène (8.09%) and germacrene-D (6.06%). The essential oil had a small antibacterial spectrum for all strains tested and the total antioxidant activity was significant. Furthermore the *D. klainea* essential oil might help to prevent oxidative damage in the human body, such as lipid peroxidation which is associated with cancer, atherosclerosis, cardiovascular deseases and diabetes. These results showed that the essential oil could be used as a potential natural antioxidant and antibacterial agent.

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