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Full Length Research Paper

Volatile constituents and antioxidant activity of *Aucoumea klaineana* Pierre essential oil

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The essential oil isolated from the resin of *Aucoumea klaineana* Pierre (Burseraceae) by Hydrodistillation was analyzed by capillary gas chromatography (GC) and combined Gas Chromatography/Mass Spectrometry (GC/MS). The analysis led to the identification of 28 components. This oil contained mainly monoterpenoids (96.06%) in which *p*-acetyl anisole is the single benzenic compound (0.18%). The predominant constituents in the essential oil were -3-carene (72.31%), *p*-cymene (3.76%), limonene (4.04%), terpinolene (6.28%) and -terpineol (4.34%). The essential oil showed antioxidant and weak DPPH radical scavenging activities and it displayed the inhibition of lipid peroxidation.

Key words: *Aucoumea klaineana,* Burseraceae, essential oil, antioxidant activity.

INTRODUCTION

At the present time, scientists have focused on the safety and the adverse effects of synthetic chemicals widely used in medicine and for food preservation. In the process of making and storage, free radicals of oxygen (Reactive Oxygen Species) cause lipid peroxidation in foods that leads to their deterioration (Mau et al., 2004). Although there are some synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), they are associated with some undesirable effects (Ito et al., 1983). Therefore, research works concerning essential oils as alternative potential antioxidant for treatment of human diseases and for food preservation were important. *Aucoumea klaineana* Pierre (Burseraceae) is largely distributed in equatorial forest from Gabon to Equatorial Guinea (Walker and Sillans, 1961). It is a tree growing to 30-40 m tall, rarely larger, with a trunk 1-2.5 m diameter above the often large basal buttresses, the major use of *A. klaineana* is in the manufacture of plywood (Chudnoff, 1984). It is a medicinal plant and the data on their traditional uses were collected by personal contact with local healers in Gabon: the roots and leaves are used to treat fever, constipation, malaria, diarrhoea and jaundice.

The resin of the plant is used to purify water and as disinfectant. Previous works have shown that *A. Klaineana* contained triterpenoid compounds (Tessier et al., 1982a) and its resin was used in the cosmetic and pharmaceutical fields (Tessier et al., 1982b) . The present study reports results of a detailed analysis of the composition of the resin essential oil and its antioxidant ability to contribute to the search for beneficial uses of this plant. To our knowledge, this is the first report of the antioxidant property of *A. klaineana* resin essential oil.

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MATERIAL AND METHODS

Plant material

The resin of *A. klaineana* Pierre was collected from Sebang Herbarium of IPHAMETRA, Libreville (Gabon) in December, 2006. Voucher specimens have been identified and deposited at the Herbarium of IPHAMETRA and laboratoire pluridisciplinaire de Chimie de l'ENS, Libreville, Gabon.

The essential oil was extracted from the resin (500 g) by hydrodistillation in a Clevenger-type apparatus for 4 h and was dried over anhydrous sodium sulphate.

Analysis

GC analysis was performed on a Hewlett-Packard HP 6890 system 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 temperature was 50°C (5 mn) rising to 300°C at a rate of 5°C / mn. Helium was used as the carrier gas at a flow rate of 1.1 ml/mn. The injection of each sample consisted of 1.0 l of oil diluted to 10% (v/v) with acetone.

GC-MS analyses were performed on a Hewlett-Packard 5973/ 6890 system operating in EI mode (70 eV), equipped with a split/splitless injector (280°C), a split ratio 1:10 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 the HP-5 MS column was 50°C (5 mn) rising to 300°C at a rate of 5°C / mn and for the HP-Innowax column, 50-250°C at a rate of 5°C / mn. Helium was used the carrier gas at a flow rate of 1.1 ml/mn. Retention indices of all compounds were determined according to Van Den DOOL approach (1963). The identification of components was based on comparison of their mass spectra with those of libraries (McLafferty and Stauffer, 1989; Adams, 2007; Joulain and König, 1998) as well as by comparison of their retention indices with literature data.

Determination of 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 (Fluka) radical solution in methanol was prepared, and then 1 ml of this solution was mixed with 3 ml of the sample solution in ethanol. Various concentrations of extracts were obtained. BHT (Sigma) was used as a positive control at 100 $g.ml^{-1}$ concentration.

After incubation for 30 min 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: %DPPH radical scavenging = [(control absorbance - sample absorbance)/control absorbance] x 100: I% radical scavenging = $[(A_{control} - A_{sample})]/ A_{control}] \times 100$.

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

Determination of antioxidant activity

The anti oxidant activity was determined according to the method described by Dapkevicius et al. (1998) . 0.5 mg of -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 -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 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:$RAA\% = (A_{sample}/A_{BHT}) \times 100$Where A_{BHT} is the absorbance of the positive control BHT and A_{sample} is the absorbance of the extract.

Statistical analysis

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

RESULTS AND DISCUSSION

Essential oil composition

The essential oil was obtained in 7.85% (w/w) yield. The constituents identified in the oil are listed in Table 1 according to their order of elution on HP-5. A total of 28 constituents were identified (96.06%) . The oil contains mainly monoterpenoids with *p*-acetyl anisole being the single benzenoid compound (0.18%). The hydrocarbons and the oxygenated compounds accounted for 88.92 and 7.14% of the constituents of the oil, respectively. Among the hydrocarbons, four monoterpenoids (86.39%) were detected of which -3-carene (72.31%), *p*- cymene (3.76%), limonene (4.04%), terpinolene (6.28%) and -terpineol (4.34%) were the most dominant. In the oxygenated fraction, menthomenthene (0.89%), mentha-1, 5-dien-8-ol(0.30%), *m*-cymen-8-ol (0.33%), *p*-cymen-8-ol (0.33%) and -terpineol (4.04%) were present as the major compounds. Due to its high content in -3- carene, *A. klaineana* Pierre from Gabon can be an interesting source of this compound and of menthol obtained from epoxidation (Leffingwell and Schackelford, 1974) both compounds are widely used in the pharmaceutical, cosmetic and food industries.

Antioxidant and DPPH radical scavenging activities

The result of DPPH free radical scavenging activity is shown in Figure 1. The essential oil obtained from resin exhibited a weak scavenging action at 100 g/ml which is also weak in comparison to the scavenging activity of a BHT 100 g/ml concentration. In the case of the linoleic acid system, the essential oil possessed strong antioxidant ability for preventing the linoleic acid oxidation, but this effect was lower than that of BHT at100 g/ml concentration (Figure 2). The strong antioxidant and weak DPPH radical scavenging activities of *A. Klaineana*

Table 1. Chemical composition of the essential oil of *Aucoumea klaineana.*

Peak	RI	Components	Percentage
1	927	- thujene	0.03%
2	934	- pinene	0.70%
3	949	fenchene	0.07%
4	972	1,2,3,4-tetramethyl benzene	0.11%
5	974	sabinene	0.46%
6	979	-pinene	0.05%
7	983	Bicyclo [3.3.1]nonane-2-one	0.03%
8	1001	Menthomenthene	0.89%
9	1012	-3-carene	72.31%
10	1018	- terpinene	0.63%
11	1021	*o* - cymene	0.08%
12	1026	*p* - cymene	3.76%
13	1031	Limonene	4.04%
14	1032	- phellandrene	0.18%
15	1034	1,8 - cineol	0.25%
16	1054	*(E)*- -ocimene	0.03%
17	1060	– terpinene	0.11%
18	1087	Terpinolene	6.28%
19	1091	*p* - cymenene	0.08%
20	1135	Mentha-2,8-dien-1-ol	0.09%
21	1145	Epoxyterpinolene	0.19%
22	1165	Mentha-1,5-dien-8-ol	0.30%
23	1178	(+)-terpinen-4-ol	0.09%
24	1183	*m* - cymen-8-ol	0.33%
25	1189	*p* - cymen-8-ol	0.33%
26	1198	- terpineol	4.34%
27	1206	Verbenone	0.12%
28	1345	*p*-acethyl anisole	0.18%

RI : Retention Indices.

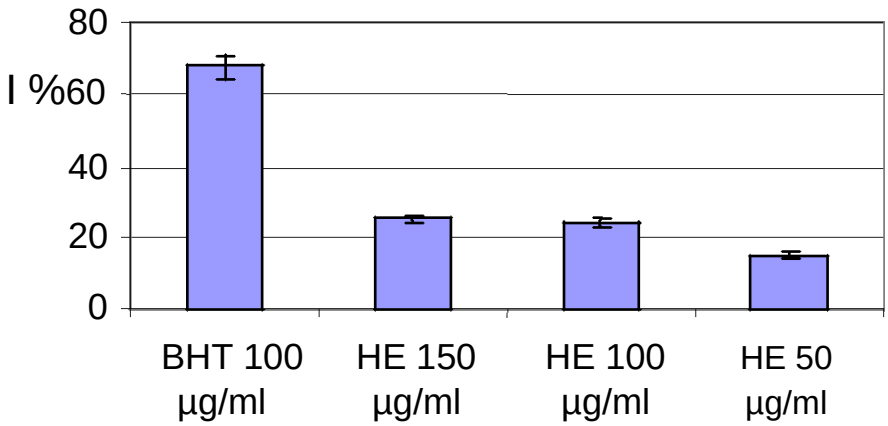

Figure 1. DPPH radical scavenging activity of *Aucoumea klaineana* Pierre essential oil.
HE: Essential oil.

essential oil can be attributed to the presence of some constituents that have antioxidant activity: terpinolene, -terpinene, -terpinene (Grassmann et al., 2003; Grassmann et al., 2005; Kim et al., 2004) p-acetylanisole (Ranalli et al., 2003).

Conclusion

The essential oil contains mainly monoterpenoids and the global antioxidant activity was significant. However the *A. klaineana* essential oil might be a potential natural agent

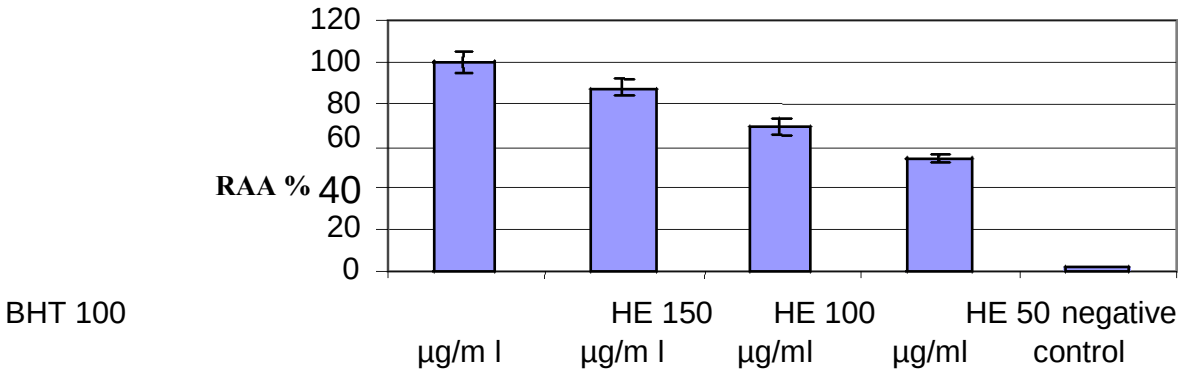

Figure 2. Antioxidant activity by- -carotene bleaching test of *Aucumea klaineana* essential oil.

HE: essential oil.

to prevent oxidative damage in the human body such as lipid peroxidation which was associated with cancer, prematuring aging, atherosclerosis and diabetes.

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