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Antifungal and Antibacterial activities of *Aucoumea klaineana* Pierre Essential Oil From Gabon

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ABSTRACT

Aucoumea klaineana Pierre (Burseraceae) is largely distributed in equatorial forest from Gabon to Equatorial Guinea. It is a medicinal plant that resin, roots and leaves are used to treat fever, constipation, malaria, diarrhea, jaundice and sexual transmissible infection treated by traditional healers. The predominant constituents in the essential oil were δ-3-carene. The antimicrobial activities of both essential oils were tested against Gram-positive and Gram -negative bacteria by using agar disc diffusion and broth microdilution methods; anticandidal effect was also tested on different strains of *Candida albicans*. The essential oil exhibited antibacterial and antifungal activities against the all strains tested. The best inhibition zones were obtained for *Shigella dysenteria* CIP 5451 and for *Candida albicans* ATCC90028. The results suggest that *Aucoumea klaineana* resin essential oil could be a natural antimicrobial agent.

Keywords: : *Aucumea klaineana, Burseraceae, Essential oil, Antibacterial and Antifungal activities*

Introduction

Aucoumea klaineana Pierre (Burseraceae) is largely distributed in equatorial forest from Gabon to Equatorial Guinea [1]. 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 [2]. It is an important medicinal plant, widely used as a home remedy for several diseases 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 [3] and its resin was used in the cosmetic and pharmaceutical fields [3].

The essential oil isolated from the resin of *Aucoumea klaineana* were 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 δ-3-carene (72.31 %), p-cymene (3.76 %), limonene (4.04 %), terpinolene (6.28%) and α-terpineol (4.34 %) [5].

The essential oil showed antioxidant and weak DPPH radical scavenging activities and it displayed the inhibition of lipid peroxidation [4].

The present study reports results of a detailed analysis of the

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antifungal and antibacterial activities of the resin essential oil ability to contribute to the search for beneficial uses of this plant. To our knowledge, this is the first report of the antimicrobial property of *A. klaineana* resin essential oil.

Materials and Methods

Plant material

The resin of *A. klaineana* Pierre was collected from Mekuê forest in Mebane Endama, Oyem (Gabon) in May, 2013. Voucher specimens have been identified and deposited at Research Laboratories in Biochemistry (LAREBIO) and Laboratory of Natural Substances and Organometallic Synthesis (LASNSOM), University of Science and Technology of Masuku, Franceville, Gabon.

The essential oil was extracted from the resin (100 g) by hydrodistillation in a Clevenger-type apparatus for 4 h and was dried over anhydrous sodium sulphate. Essential oils were stored in airtight containers in a refrigerator at 4 °C. Essential oil yield were calculated to the weight of the plant material before distillation (expressed in percent, W/w of the dry vegetable material).

Microbial strains

The bacterial strains used were: *Bacillus cereus* LMG 13569, *Enterococcus faecalis* CIP 103907, *Escherichia coli* NCTC 11602, *Listeria innocua* LMG 1135668, *Salmonella enterica* CIP 105150, *Shigella dysenteria* CIP 5451, *Staphylococcus aureus* ATCC 9244, *Staphylococcus camorum* LMG 13567, *Proteus mirabilis* CIP 104588, *Enterococcus faecalis* (n=10)., *Pseudomonas aeruginosa* (n=10), *Staphylococcus aureus* (n=5) and *Streptococcus pyogenes* (n=3).

The fungal strains used were: *Candida albicans* ATCC 10231, *Candida albicans* ATCC 90028 and *Candida albicans* (n=10).

Antimicrobial assay

A. Disc diffusion method

The agar disc diffusion method was employed for the screening of antimicrobial activities of the essential oils [5]. The test was performed in sterile Petri dishes (90 mm diameter) containing solid and sterile Mueller-Hinton agar medium (Becton Dickinson, USA) for bacterial strains and Sabouraud-Dextrose agar for the yeasts. The oil absorbed on sterile paper discs (5 µl per Whatman disc of 6 mm diameter) were placed on the surface of the media, previously inoculated with 0.1 ml of microbial suspension. One filter paper disc was placed per Petri dish in order to avoid a possible additive activity, exhibited via the vapour phase, of the components from more than one disc. Every dish was sealed with laboratory film to avoid evaporation, then incubated aerobically at 30 or 37 °C for 24 h. Tetracycline (30 UI), ticarcilline (75 mg), fluconaz1 (100 mg) and griseofulvin (100 mg) were used as standard antibiotics. Results were interpreted in terms of diameter of the inhibition zone. All tests were performed in triplicate.

B. Microdilution method

A microdilution broth susceptibility assay was used, as recommended by NCCLS, for the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) [6]. All tests for bacteria were performed in Mueller-Hinton Broth (Becton Dickinson, USA) supplemented with Tween-80 detergent (final concentration of 0.5 % (v/v)), and for the yeasts Sabouraud-Dextrose broth was used. A serial doubling dilution of the essential oil was prepared in 96 wells plates over the range 0.25 – 32 % (v/v). Overnight cultures of each strain were prepared in Nutrient broth (Diagnostic Pasteur, France) and the final concentration in each well was adjusted to 5×10^5 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 or 37°C and MICs were determined. The MIC was defined as the lowest concentration of the essential oil at which the microorganism tested does not demonstrate visible growth. To determine MBCs, 10 ml broth was taken from each well and inoculated in Mueller-Hinton Agar (Becton Dickinson, USA) for 24 h at 30 or 37°C. The MBC was defined as the lowest concentration of the essential oil at which 99.99 % or more of the initial inoculum was killed.

Statistical analysis

The data were analyzed with Student's t-test or one-way ANOVA followed by Bonferroni test (GraphPad Prism 5.01; GraphPad Software Inc., San Diego, USA). The criterion for statistical significance was taken as $p < 0.05$.

Results and Discussion

Antimicrobial activity

The essential oil was obtained in 7.85 % (w/w) yield. The antimicrobial activity of *A. klaineana* resin essential oil was evaluated against a set of 16 microorganisms and their potency was assessed qualitatively and quantitatively by the presence or absence of inhibition zones diameters (ZDs) and MIC values. The correlation between two different screening methods examined was generally larger ZDs correlated with lower MICs. The results in table 2 showed that the growth of bacterial species and fungal species tested was significantly reduced by the essential oil of *Aucumea klaineana* and in many cases the growth of some bacterial and fungal species was completely inhibited. It was also interesting to find that the inhibition effects of the oil against some fungal strains and bacterial strains were higher than that of reference drugs particularly for *Pseudomonas aeruginosa*.

This activity was more marked against the Gram-positive bacteria. *Shigella dysenteria* CIP 5451 (50 mm), *Enterococcus faecalis* CIP 103907 (45 mm), *Bacillus cereus* LMG 13569, *Staphylococcus camorum* LMG 13567 and *Escherichia coli* CIP 11602 for the reference strains and *Pseudomonas aeruginosa* (40 mm) for hospital strains, were the most sensitive

Table 1. Diameter of inhibition zone (mm) of *Aucoumea klaineana* essential oil

Reference strains	DD, Diameter of disk diffusion			
	Origin	*A klaineana*	Te[b]	Ti[b]
Bacillus cereus LMG 13569	LMG	40	18	50
Enterococcus faecalis CIP 103907	CIP	45	19	30
Escherichia coli CIP NCTC 11602	CIP	40	22	8
Listeria innocua LMG1135668	LMG	30	14	50
Salmonella enterica CIP 105150	CIP	32	16	50
Shigella dysenteria CIP 5451	CIP	50	21	31
Staphylococcus aureus ATCC 9244	ATCC	48	17	48
Staphylococcus camorum LMG 13567	LMG	40	20	nd[c]
Proteus mirabilis CIP 104588	CIP	9	15	nd[c]
Clinic strains				
Enterococcus faecalis (n=10)	Foecal	24±2	20	28
Pseudomonas aeruginosa (n=10)	Vaginal liquid	40±3	21	nd[c]
Staphylococcus aureus (n=5)	Vaginal liquid	28±4	21	27
Streptococcus pyogenes (n=3)	Vaginal liquid	24±2	20	24.
Fungal strains			Fluco	Griseo
Candida albicans ATCC 10231	ATCC	23	13	15
Candida albicans ATCC 90028	ATCC	20	13	10
Candida albicans (n=10)	Uro-vaginal	35±3	9	11

Each values are means ± standard deviation of three separate experiments. Te[b]: tetracycline , Ti[b] : ticarcilline., A klaineana, Aucoumea klaineana, , Fluco. Fluconazol, Griseo. Griseofluvine, cnd: non determined

microorganism to the essential oils while *Proteus mirabolis* 104588 CIP (9 mm) was the most resistant. The other strains had sensitivities between 30-38 mm. Following the results in table 1, the different strains were more sensitive to *A. klaineana* resin essential oil than tetracycline. The most important information was that *A. klaineana* resin essential oil exhibited more activity on *S. dysenteria* CIP 5451 (50 mm), *E. faecalis* CIP 103907 (45 mm), *B. cereus* LMG 13569, *S. camorum* LMG 13567, *E. coli* CIP 11602 and Ticarcilline.

The essential oil was tested against *Candida albicans* as pathogenic fungal species in human body and compared with fluconazole and griseofulvin.

The essential oil exhibited the best antifungal activity for *Candida albicans* (Table 1). Clinic origin *C. albicans* was more sensitive to *A. klaineana* resin essential (23 mm) than reference *C. albicans* strains [*C. albicans* ATCC 10231 (20) and *C. albicans* ATCC 90028 (28)]. It was also interesting to find that the inhibition effect of the essential oil against *C. albicans* (35 mm) were higher than that of fluconazole (*C. albicans*, 9 mm) and griseofulvin (*C. albicans*, 11 mm).

In previous works, essential oils which had chemical compositions and major components such as: δ-3-carene [8], linalool, α-pinene, β-pinene, limonene, 1,8-cineole [8] exhibited antibacterial and antifungal activities. These reports are compatible with our results in the present study.

The MICs, MBCs and MFCs of *A. klaineana* resin essential oil for all the strains tested are presented in Table 2. The essential oil failed to inhibit.

The MICs, MBCs and CMFs of resin essential oil *Aucoumea klaineana* microorganisms were shown in Table 2. Strong activity was confirmed by the determination of MIC and MBC ranging from 1 to 8 %.

The essential oil shows a strong antibacterial activity with MIC and MBC equal to 1 % on *Bacillus cereus* LMG 13569, *Escherichia coli* CIP 11602 and *Staphylococcus aureus* ATCC 9244. *Salmonella enterica* CIP 105150, *Staphylococcus camorum* LMG 13567 and *Enterococcus faecalis* are susceptible with MIC and MBC 2 %. The oil is bactericidal with MIC and MBC equal to 4 % of *Enterococcus faecalis* CIP 103907, *Listeria innocua* LMG 1135668, *Proteus mirabolis* CIP 104588 and *Shigella dysenteriae* CIP 5451.

The oil has the lowest bactericidal activity and appears to be less active with MIC greater than 8% on hospital strains *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Conclusion

The development of natural antimicrobial agents will help to decrease resistance of pathogenic microorganisms. The *Aucumea klaineana* essential oil had wide spectra of antimicrobial activities. Its inhibitory effects are high in comparison to tetracycline, fluconazole and griseofulvin. These results indicate that the essential oil of *Aucumea klaineana* could be used as antimicrobial agent and antioxidant agent for the therapy of human being and for food preservation respectively. However the *A. klaineana* essential oil might be a potential natural agent to prevent oxidative damage in the human body.

Table 2. Minimum inhibitory concentration, minimum bactericidal concentration, minimum fungicidal concentration data (% v/v) of *Aucoumea klaineana* essential oil obtained by microdilution method.

Reference strains	Origin	CMI	CMB
Bacillus cereus LMG 13569	LMG	1	1
Enterococcus faecalis CIP 103907	CIP	4	4
Escherichia coli CIP NCTC11602	CIP	1	1
Listeria innocua LMG1135668	LMG	4	4
Proteus mirabolis 104588 CIP	CIP	4	4
Salmonella enterica CIP105150	CIP	2	2
Shigella dysenteria CIP 5451	CIP	4	4
Staphylococcus aureus ATCC9244	ATCC	1	1
Staphylococcus camorum LMG13567	LMG	2	2
Souches cliniques			
Enterococcus faecalis	Fécale	2	2
Pseudomonas aeruginosa	liquide vaginal	16	16
Staphylococcus aureus	liquide vaginal	8	8
Streptococcus pyogenes	liquide vaginal	16	16

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