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Full Length Research Paper

Chemical composition, antioxidant and antimicrobial properties of the essential oil of *Dacryodes edulis* (G. Don) H. J. Lam from Gabon

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The essential oil obtained by hydrodistillation from the resin of Dacryodes edulis (G. Don) H. J. Lam was simultaneously analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC -MS). Twenty four components were identified in the essential oil and the main components were sabinene (21.8%), terpinene-4-ol (19.8%), □-pinene (17.5%) and p-cymene (11.3%), respectively. The antioxidant capacity of the essential oil was examined using an in vitro radical scavenging activity test and -carotene-linoleic acid assays. In the 2.2-Diphenylpicrylhydrazyl (DPPH) test system, the IC50 value of D. edulis oil was 68.5 ± 2.29 µg/ml. In the -carotene-linoleic acid test system, oxidation of linoleic acid was effectively inhibited by D. edulis (70.0%). The oil was less effective than butylated hydroxytoluene (BHT). Furthermore, the essential oil was evaluated for its antimicrobial activity using disc diffusion and microdilution methods. The essential oil showed better activity against bacterial species than against yeast.

Key words: *Dacryodes edulis*, Burseraceae, essential oil, antimicrobial activity; antioxidant activity; gas chromatography-mass spectrometry.

INTRODUCTION

Dacryodes edulis, named “African pear” or “Safou”, is a tropical tree producing a consumable fruit, which softens when heated and then would be eaten with cassava or as a dessert. The native area of Safou extends from Sierra Leone to Uganda to the east and to Angola to the south. It is an attractive tree, usually 8 - 12 m in height, but sometimes reaching 20 - 25 m in dense forest stands. Safou belongs to the family of Burseraceae. The wounded bark exudes a limpid resin that becomes opaque while solidifying. The burning resin releases a strong odour (Raponda and Sillans, 1961).

D. edulis has a long history of use in folk medicine. Traditional healers in Nigeria and in the Democratic Republic of Congo use the plant to treat various infections. It is used in traditional medicine as a remedy for parasitic skin diseases, jigger, mouthwash, tonsillitis and drepanocytosis (Burkill, 1994; Mpianaa et al., 2007).

Essential oils from different parts of *D. edulis* have been isolated and analyzed. Essential oil of untreated, boiled and roasted fruits contains many constituents among which α-pinene, β-pinene, myrcene, limonene and sabinene were found to be the main compounds (Jirovetz et al., 2003). The stem bark essential oil contains predominantly terpinen-4-ol, α-thujene and α- pinene, whilst α-phellandrene is the major component of the root bark oil. β-caryophyllene is a dominant constituent of the leaf oil (Onocha et al., 1999). The resin has been reported to yield a peppery essential oil that is rich in sabinene, β-phellandrene and limonene (Burkill, 1994).

Information concerning *in vitro* antioxidant and antimicrobial activities of the essential oil from the resin of *D. edulis* has not been reported earlier. The aim of this study was to evaluate the *in vitro* antioxidant and antimicrobial properties of the essential oil from resin of *D. edulis* from Gabon.

MATERIALS AND METHODS

Plant material

The resin of *Dacryodes edulis* was collected in December 2006 from the Herbarium Sebang of IPHAMETRA in Libreville (Gabon). A voucher specimen has been deposited at the Herbarium of

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IPHAMETRA and the Laboratoire Pluridisciplinaire des Sciences (LAPLUS) de l'Ecole Normale Superieure de Libreville Gabon.

Essential oil isolation and analysis

The resins of *Dacryodes edulis* G. Don were subjected to hydrodistillation for 3 h using a Clevenger- type apparatus. The oil obtained was dried over anhydrous sodium sulfate, filtered and stored in a sealed vial in the dark at 4°C before analysis and bioassay tests.

GC analysis was performed on a Hewlett Packard 6890 gas chromatograph equipped with a FID and HP-5ms capillary column (bonded and cross-linked 5% phenyl-methylpolysiloxane 30 m· 0.25 mm i.d., film thickness 0.25 lm). Injector and detector temperatures were set at 250 and 300ëC, respectively. The oven temperature was held at 50ëC for 5 min, then programmed to 240ëC at a rate of 4ëC /min. Helium was the carrier gas, at a flow rate of 1 ml/min. Diluted samples (1/100 in acetone, v/v) of 1.0 µl were injected manually and in the splitless mode. Quantitative data were obtained electronically from FID area percent data.

GC-MS analysis of the essential oil was performed under the conditions given above with GC, using a Hewlett Packard 6890 gas chromatograph equipped with a Hewlett Packard 5973 mass selective detector in the electron impact mode (70 eV). Mass range was from 35 to 450 m/z. *n*-Alkenes were used as reference points in the calculation of the Kovats Indices (K.I.). Identification of the oil components was done by comparison of their relative retention index and mass spectra with those of NIST library data of the GC-MS system and literature data (Adams, 2001).

Antioxidant activity

2,2-Diphenylpicrylhydrazyl (DPPH) assay

The hydrogen atoms or electron-donating ability of the corresponding extracts and butylated hydroxytoluene (BHT) was determined from the bleaching of purple-coloured methanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent (Tepe et al. , 2004, 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. 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 percent DPPH radical scavenging, which is calcu-lated with the equation: %DPPH radical scavenging = [(control absorbance- sample absorbance)]/control absorbance] × 100. The 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.

Beta-carotene-linoleic acid assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxide formation from linoleic acid oxidation (Tepe et al., 2005). A stock solution of Beta-carotene-linoleic acid mixture was prepared as follows: 0.5 mg of -carotene was dissolved in 1 ml of chloroform (HPLC grade), and 25 µl of linoleic acid and 200 mg of Tween-40 were added as emulsifier since -carotene is not water soluble. Chloroform was completely evapored using a va-cuum evaporator. Then, 100 ml of oxygen-saturated distilled water was added with vigorous shaking at a rate of 100 ml/min for 30 min; 2500 µl of this reaction mixture was dispensed into test tubes, and 350 µl volumes of extracts, prepared in 2 g/l concentrations, were added. The emulsions were 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 that of BHT and the blank. Tests were carried out in triplicate.

Microbial strains

The essential oil of resin from *Dacryodes edulis* was tested against a panel of microorganisms, including reference and clinical strains. Reference strains were *Bacillus cereus* LMG 13569, *Enterococcus faecalis* CIP 103907, *Escherichia coli* CIP 105182, *Listeria innocua* LMG 113568, *Salmonella enterica* CIP 105150, *Shigella dysenteria* CIP 5451, *Staphylococcus aureus* ATCC 9244, *Proteus mirabilis* 104588 CIP, *Staphylococcus camorum* LMG 13567, *Candida albicans* ATCC 10231 and *Candida albicans* ATCC 90028.

Clinical strains of *Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes* and *C. albicans* were isolated at the Laboratoire de Biologie Médicale Saint Camille de Ouagadougou.

Disc diffusion method

The agar disc diffusion method was employed for the screening of antimicrobial activities of the essential oils (NCCLS, 1997). 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 µg), fluconaz1 (100 µg) and griseofulvin (100 µg) were used as standard antibiotics. Results were interpreted in terms of diameter of the inhibition zone: (-): < 6 mm; (+): 6 – 10 mm; (++): 11 – 15 mm; (+++): ≥ 16 mm. All tests were performed in triplicate.

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) (NCCLS, 1999). 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 + Tween-80 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. The number of surviving

Table 1. Chemical composition of the essential oil of *D. edulis*.

Pics	K.I. HP-5ms column	Compounds	Percentage
1	927	αthujene	1.56
2	935	α–pinene	17.47
3	951	camphene	0.24
4	975	sabinene	21.77
5	979	β-pinene	4.27
6	1001	menth-3-ene	0.37
7	1007	α–phellandrene	0.22
8	1009	δ-3-carene	0.23
9	1018	α terpinene	1.22
10	1026	para cymene	11.29
11	1031	limonene	5.72
12	1032	β-phellandrene	0.99
13	1034	1,8-cineol	0.68
14	1060	γ–terpinene	5.84
15	1072	cis hydrate sabinene	1.08
16	1086	terpinolene	1.08
17	1102	trans hydrate sabinene	0.4
18	1127	cis para menth-2-en-1-ol	0.4
19	1145	trans paramenth-2-en-1-ol	0.37
20	1184	terpinene-4-ol	19.79
21	1189	Para cymene-8-ol	0.13
22	1197	α–terpineol	3.01
23	1211	trans piperitol	0.2
24	1257	Piperitone	0.22

organisms was determined by viable count.

RESULTS AND DISCUSSION

This is the first report on the composition of resin oil from *D. edulis* growing in Gabon. Hydrodistillation of the resin oil of *D. edulis* gave the limpid oil with a specific odour in 0.68% (v/w) yield. The volatile samples were analyzed simultaneously by GC and GC-MS methods. The list of compounds detected, together with their relative percentages and relative retention indices, are given in Table 1 in order of their elution on a HP-5ms capillary column. Twenty-four compounds were identified and constituted 98.5% of the total oil. Sabinene (21.8%), terpinene-4-ol (19.8%), α-pinene (17.5%) and *p*-cymene (11.3%) were the major components comprising the 70.3% of the essential oil. While under steam distillation, the resin has been reported to yield a peppery essential oil rich in. This chemotype is different from sabinene, β- phellandrene and limonene one reported by Burkill (1994).

The essential oil was subjected to screening for their possible antioxidant activity by two complementary test systems, namely DPPH free radical scavenging and β-carotene-linoleic acid assays. BHT was used as positive control in both test systems. In the DPPH test system, the IC_{50} value of *D. edulis* oil was 68.5 ± 2.3 µg/ml. In the β-carotene-linoleic acid system, oxidation of linoleic acid was effectively inhibited by *D. edulis* (70.0%) . When com-pared to BHT, the oil was less effective. This could due to the chemical composition of the essential oil, as the essential oil contained mainly monoterpene hydrocarbons such as sabinene, terpinene-4-ol, α-pinene, *p*-cymene, γ-terpinene, limonene, β-pinene and α- terpineol (Table 1). Indeed, these compounds are known to possess a weak antioxidant activity (Tepe et al., 2005).

The antimicrobial activity of the essential oil of *D. edulis* against 16 species of microorganisms by the disc diffusion method and the broth microdilution is reported in Table 2. The results showed that the essential oil of *D. edulis* had an antimicrobial activity against all tested microorganisms. In all cases the MIC was equivalent to the MBC, indicating a bactericidal action of the oil of *D. edulis* G. Don. The essential oil showed better activity against bacterial species than against yeast. It has frequently been reported that Gram-positive bacteria are more sensitive to plant oil and their components than Gram-negative bacteria (Consentino et al., 1999; Karaman et al., 2003; Sahin et al., 2002). However, the

Table 2. Antimicrobial activity from resin essential oil of *D. edulis* from Gabon.

Reference strains	Antibiotics		*Dacryodes edulis*		
	Tetracycline	Ticarcilline	DD[a]	MIC[b]	MBC[b]
Bacterial strains					
Bacillus cereus LMG 13569	+++	+++	+++	1	1
Enterococcus faecalis CIP 103907	+++	+++	+++	1	1
Escherichia coli CIP 105182	+++	+	+++	1	1
Listeria innocua LMG1135668	++	+++	+++	4	4
Proteus misrabilis 104588 CIP	++	NT	+++	4	4
Salmonella enterica CIP 105150	++	+++	+++	1	1
Shigella dysenteria CIP 5451	+++	+++	+++	1	1
Staphylococcus aureus ATCC 9244	+++	+++	+++	1	1
Staphylococcus camorum LMG 13567	+++	NT	+++	2	2
Enterococcus faecalis (Clinical isolate)	+++	+++	+++	2	2
Pseudomonas aeruginosa (Clinical isolate)	+++	NT	+++	16	16
Staphylococcus aureus (Clinical isolate)	+++	+++	+++	8	8
Streptococcus pyogenes (Clinical isolate)	+++	+++	+++	8	8
Fungal strains	Fluconaz1	Griseofulvin		MIC	MIF
Candida albicans ATCC 10231	++	++	+++	8	8
Candida albicans ATCC 90028	+	+	+++	16	16
Candida albicans (Clinical isolate)	-	+	+++	8	8

[a] Tested at a concentration of 5 µl/disc

[b] Values given as percentage

NT: Not tested

Results of disc diffusion (DD) method were interpreted in terms of diameter of inhibition zone: (-): <6 mm; (+): 6 – 10 mm ; (++): 11 – 15 mm; (+++): ≥ 16 mm

results in this study showed that essential oil of *D. edulis* did not have selective antimicrobial activities on the basis of the cell well differences of bacterial microorganisms. Based on these results, it is possible to conclude that the essential oil has a stronger and broader spectrum of anti-microbial activity. This result may be explained by the high content of terpinene-4-ol (19.8 %) and α- pinène (17.4%) in the essential oil of *D. edulis* analyzed in the present study. Antibacterial and antifungal activities of these compounds have been reported in previous studies (Carson and Riley, 1995; Cox et al., 2001; Inouye et al., 2001).

Since there are no previous reports on the antioxidative and antimicrobial activities of the plant oil presented here, our study may be considered as the first report on the antioxidant and antimicrobial properties of the essential oil of *D. edulis.* Our work showed that essential oil of *D. edulis* possesses potential antimicrobial activity.

This result may indicate that essential oil of *D. edulis can* be used as natural preservatives in food against the well- known causal agents of foodborne diseases and food spoilage.

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