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Volatile Components, Antioxidant and Antimicrobial Properties of the Essential Oil of *Dacryodes edulis* G. Don from Gabon

[1]J. Koudou, [2]P. Edou, [3]L.C. Obame, [3]I.H. Bassolé, [4]G. Figueredo,
[5]H. Agnaniet, [6]J.C. Chalchat and [3]A.S. Traore
[1]Laboratoire de Chimie des Substances Naturelles, Faculté des Sciences,
Université de Bangui, BP 908 Bangui, République Centrafricaine
[2]Ecole Normale Supérieure, Laboratoire Pluridisciplinaire de Chimie, BP 17009 Libreville, Gabon
[3]Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles,
Laboratoire de Microbiologie et de Biotechnologie,
Université de Ouagadougou, 03 BP 7131 Ouagadougou, Burkina Faso
[4]LEXVA Analytique, 460 rue du Montant, 63110 Beaumont, France
[5]USTM, Faculté des Sciences Laboratoire de Chimie, BP 769, Franceville, Gabon
[6]Laboratoire de Chimie des Hétérocycles et des Glucides, Chimie des Huiles Essentielles
Les Cézeaux, 63177 Aubière, France

Abstract: The resin oil obtained by hydrodistillation from *Dacryodes edulis* G. Don growing in Gabon was analyzed by GC and GC/MS. The major constituents in the essential oil were sabinene (21.76%), terpinene-4-ol (19.79%), α-pinene (17.47%) and p-cymene (11.29%). The *in vitro* antioxidant activity was investigated with two methods: 2, 2-diphenylpicrylhydrazyl radical (DPPH) scavenging essay and β-carotene bleaching test. Butylated hydroxyltoluene was employed as positive control. The essential oil showed antioxidant and DPPH radical scavenging activities and it displayed the inhibition of lipid peroxidation. Furthermore, the antimicrobial activity of essential oil was evaluated using a broth microdilution method. *Dacryodes edulis* essential oil exhibited antibacterial activity but it was unable to inhibit the growth of fungal species tested.

Key words: *Dacryodes edulis*, Burseraceae, essential oil, antioxidant, antibacterial

INTRODUCTION

Dacryodes edulis G. Don (Burseraceae) is a tropical African species usually up to 8 and 18 m or sometimes reaching 45 m in height and producing an edible fruit named Safou. The scars of bark exude a limpid resin which becomes opaque while solidification and the resin spreads a strong odor. The fruit can take various forms and sometimes reach 15 cm in length (Kengue, 1994).

Traditional healers in Nigeria and Cameroon use the plant to treat various diseases such as body aches, cough, malaria (Ibe and Nwufo, 2005). In Gabon, *D. edulis* is used as a remedy for dermatitis and tonsillitis (Personal investigation).

Aromatherapy is now considered to be another alternative way in healing people and the therapeutic values of aromatic plants lie in their volatile constituents such as monoterpenoids, sesquiterpenoids and phenolic compounds that produce a definite physiological action on the human body (Bruneton, 1987).

To our literature survey, no study concerning the chemical composition and pharmacological properties of the Gabon species essential oil has been done before. The present research report results of volatile constituents, antioxidant and antimicrobial activities of *Dacryodes edulis* with the aim to contributing to the search for beneficial uses of this plant.

MATERIALS AND METHODS

Chemicals: DPPH (2, 2-diphenylpicrylhydrazyl) radical was obtained from Fluka, BHT (Butylhydrazyltoluene) from Sigma, tetracycline and ticarcilline (Bio-Rad Marnes-la coquette-France), fluconazole and griseofulvine (Bio-Rad-la coquette, France), sodium sulphate and acetone from prolabo, β-carotene, linoleic acid, tween 80 from Merck, all the solvents were of analytic grade.

Corresponding Author: J. Koudou, Laboratoire de Chimie des Substances Naturelles, Faculté des Sciences, Université de Bangui, BP 908, Bangui République Centrafricaine Tel: +22650368146 Fax: +22650368573

Plant material: The resins of *Dacryodes edulis* were collected in December 2006 from Sebang Herbarium of IPHAMETRA, Libreville, Gabon. A voucher specimen has been identified and deposited at the Sebang Herbarium of IPHAMETRA and at the Laboratoire Pluridisciplinaire des Sciences Ecole Normale Superieure de Libreville, Gabon. The resins (500 g) were hydrodistillated for 3 h using a Clevenger-type apparatus. The essential oil was dried, after decantation, over anhydrous sodium sulphate.

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

GC/MS analyses were carried out on a Hewlett-Packard 5973/6890 system operating in EI mode (70 eV) using two different columns: a fused silica HP-5 MS capillary column (25 m×0.25 mm, film thickness 0.25 m) and a HP-Innowax capillary column (60 m×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^{-1}. Helium was use as the carrier gas at a flow rate of 1.1 mL min^{-1}. 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; McLafferty and Stauffer, 1989; Van Den Dool and Kratz, 1963).

2, 2-diphenylpicrylhydrazyl (DPPH) assay: The free radical scavenging activity of essential oil was determined according to the method described by Burits and Bucar (2000).

β-carotene-linoleic acid essay: The antioxidant ability of the essential oil was determined according to the method previously described by Dapkevicius *et al*. (1998).

Micro organisms: The reference microbial strains used 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 mirabolis* 104588 CIP, *Staphylococcus camorum* LMG 13567, *Candida albicans* ATCC 10231 and *Candida albicans* ATCC 90028.

Clinical microbial strains: *Enterococcus faecalis*, *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes* and *C. albicans*. They were kindly provided by the St. Camille Hospital of Ouagadougou, Burkina Faso.

Antimicrobial activity essay: A broth microdilution method was used to determine the Minimum Inhibitory Concentration (MIC), the Minimum Bactericidal Concentration (MBC) and the Minimum Fungicidal Concentration (MFC) (Bassole *et al*., 2003). 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 at 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 5×10^5 cfu mL^{-1} 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^{-1} of bacterial suspension were removed from each well 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). All tests 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 (Duncans multiple range test).

RESULTS AND DISCUSSION

Chemical analyses: The hydrodistillation of the resins of *Dacryodes edulis* gave a mobile oil in 6.78% yield (w/w). The compounds identified in the oil are shown in Table 1. A total of 29 components were identified (98.52%). The oil contains exclusively monoterpenoids with hydrocarbons (72.25%) being predominant. The oxygenated compounds accounted for (25.11%) of the constituents of the oil. Among the hydrocarbons, five monoterpenoids were dominant: α-pinene (17.47%),

Table 1: Chemical composition of the essential oil of *Dacryodes edulis* G. Don

Peak	RI	Constituents	%
1	927	α-Thujene	1.55
2	935	α-Pinene	17.47
3	951	Camphene	0.24
4	975	Sabinene	21.76
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	p-cymene	11.29
11	1031	Limonene	5.72
12	1032	β-phellandrene	0.99
13	1034	1,8-cineole	0.68
14	1060	γ-terpinene	5.84
15	1072	Cis sabinene hydrate	1.08
16	1086	Terpinolene	1.08
17	1091	p-cymenene	tr
18	1102	Trans sabinene hydrate	0.39
19	1119	β-thujone	tr
20	1127	Cis p-menth-2-en-1-ol	0.40
21	1138	Terpinen-1-ol	tr
22	1142	Trans sabinol	tr
23	1145	Trans p-menth-2-en-1-ol	0.37
24	1148	Camphre	tr
25	1184	Terpinen-4-ol	19.79
26	1189	p-cymene-8-ol	0.13
27	1197	α-terpineol	3.01
28	1211	Trans piperitol	0.20
29	1257	Piperitone	0.22

RI: Retention Indices according to HP-5 column elution, tr: Trace percentage<0.1%

Table 2: Minimum inhibitory concentration, minimum bactericidal concentration data (%v/v) obtained by microdilution method.

Reference strains	Origin	MIC (%)	MBC (%)
Bacillus cereus LMG13569	LMG	1	1
Enterococcus faecalis CIP103907	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 CIP5451	CIP	4	4
Staphylococcus aureus ATCC9244	ATCC	1	1
Staphylococcus camorum LMG13567	LMG	2	2
Hospital strains			
Enterococcus faecalis	Faecal	2	2
Pseudomonas aeruginosa	Vaginal liquid	16	16
Staphylococcus aureus	Vaginal liquid	8	8
Streptococcus pyogenes	Vaginal liquid	16	16
Fungal strains		MIC	MFC
Candida albicans ATCC10231	ATCC	8	8
Candida albicans ATCC90028	ATCC	16	16
Candida albicans (n = 2)	Uro-vaginal liquid	8	8

Each value represents mean of three different observations

sabinene (21.76%), p-cymene (11.29%), γ-terpinene (5.84%), limonene (5.72%). Among the oxygenated compounds (26.27%), four monoterpenoids were present, with 1,8-cineole (0.68%), cis-sabinene hydrate (1.08%), terpinen-4-ol (19.79%) and α-terpineol (3.01%) as the major compounds. Finally, no phenolic compound has been detected in this essential oil.

Antioxidant and DPPH free radical scavenging activities : The result of DPPH free radical scavenging activity is shown in Fig. 1. The essential oil obtained from resin exhibited a better scavenging effect at 100 μg mL^{-1}, however it showed a weak scavenging activity in comparison to the activity of a BHT 100 μg mL^{-1} concentration. In the case of the linoleic acid system, the essential oil possessed a good antioxidant capacity for preventing the linoleic acid per oxidation, but this effect was significantly lower than that of BHT at 100 μg mL^{-1} (Fig. 2). The high antioxidant and DPPH radical scavenging activities of *Dacryodes edulis* essential oil can be attributed to the presence of some major components that have antioxidant activity: α-pinene, (Houghton, 2004) and terpinen-4-ol (Lee and Shibamoto, 2001).

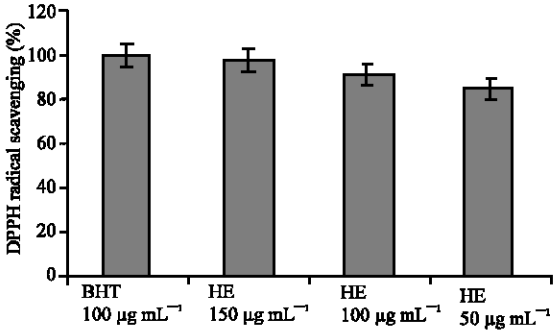

Fig. 1: DPPH radical scavenging activity of *Dacryodes edulis* essential oil

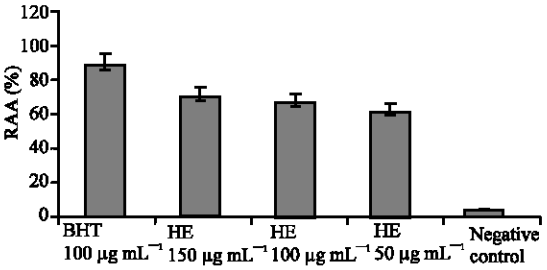

Fig. 2: Antioxidant activity by-β-carotene bleaching test of *Dacryodes edulis* essential oil

Antimicrobial activity: In this present study, MICs, MBCs and MFCs varied from 1 to 16% for all micro organisms tested (Table 2). In order to elucidate the anti microbial effect, MBC/MIC ratios were calculated. When the ratio value was lower than 1, essential oil exhibited a bactericidal effect. The better MICs were observed with *Bacillus cereus* LMG13569, *Escherichia coli* CIP NCTC11602, *Salmonella enterica* CIP105150, *Staphylococcus aureus* ATCC9244, *Staphylococcus camorum* LMG13567, *Enterococcus faecalis*. However, in the most cases the MIC was equivalent to the MBC and indicated a bactericidal action of the oil. The essential oil was bactericidal for *Bacillus cereus* LMG13569, *Escherichia coli* CIP NCTC11602, *Staphylococcus aureus*

ATCC9244 and *Enterococcus faecalis*. The most resistant strains with high MIC and MBC were *Pseudomonas aeruginosa* and *Streptococcus pyogenes*.

However, The essential oil possessed no antifungal action, *Candida albicans* ATCC90028 was the most resistant. The antibacterial activity of *Dacryodes edulis* may be attributed to the presence of the main components in the resin essential oil: p-cymene, γ-terpinene (Sonboli *et al*., 2005).

CONCLUSION

These data have provided a wealth information on the essential oil composition, antioxidant and antibacterial activities of the *Dacryodes edulis* G. Don resin. The essential oil is bactericidal for certain strains tested, its antibacterial spectrum is middle and the oil possesses a good antioxidant activity. *Dacryodes edulis* may help to prevent oxidative damage in the human body, such as lipid peroxidation which is associated with cancer, prematuring aging, atherosclerosis and diabetes. In other hand, the essential oil of *Dacryodes edulis* may be use in meat and poultry products to prevent or slow oxidative rancidity of fats that cause browning and deterioration. These results show that the essential oil could be used as a potential natural antioxidant and antibacterial agent.

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