MONOTERPENES, SESQUITERPENES AND FATTY ACIDS FROM *JULOCROTON TRIQUETER* (EUPHORBIACEAE) FROM CEARA - BRAZIL

SIDNEY G. DE LIMA[1], JOSÉ M. M. NETO[1], ANTÔNIA M. G. LOPES CITO[1], JOSÉ G. M. DA COSTA[2], FRANCISCO A. M. REIS[3]*

[1*]*Departamento de Química, Universidade Federal do Piauí, Campus Universitário Ministro Petrônio Portella – Bairro Ininga – Teresina – PI. CEP: 64.049-550.* [2]*LPPN – URCA. Crato - CE, Brasil.*
[3]*Instituto de Química – Unicamp – Campinas – SP.*
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ABSTRACT

In this study the volatile constituents from leaves and fruits of *Julocroton triqueter* (Lam.) Didr. (Euphorbiaceae), a medicinal plant from northwest Brazil, were investigated by GC/MS. Twenty one compounds, which represent about 96% of the total constituents of the essential oil, were identified. Caryophyllene oxide, humulene epoxide II, *trans*-caryophyllene, occidentalol, *α*-humulene in the fruit and *cis*-carvyl acetate, spathulenol, *cis*-carveol, *trans*-caryophyllene, *trans*-carvyl acetate and α-humulene in the leaves were the principal components. From one fraction of the hexane extract of the fruits a mixture of fatty acids were identified as: dodecanoic, tetradecanoic, hexadecanoic, 9-octadecenoic, eicosanoic, trieicosanoic and tetracosanoic acids. The partial analysis of the non-volatile constituents (hexanic fraction) from fruit allowed the isolation and characterization of tetracosan-1-ol (identify by ^{1}H and ^{13}C NMR, FTIR).

Keywords: *Julocroton triqueter*, volatile constituents, Euphorbiaceae, fatty acid

INTRODUCTION

The Euphorbiaceae family presents about 7.500 species distributed mainly in tropical areas with larger dispersion centers in America and Africa[1-2]. It has great importance not only for the number of species but also for its economical implications related to medicinal and cosmetic industry as well as for its toxicological aspects[3-6]. Many Euphorbiaceae are well known in different parts of the world as toxic and/or medicinal. The high diversity of the described effects is a reflex of the high chemical diversity of this plant group[3]. The genus *Julocroton* (Euphorbiaceae) has many representative species in Brazil and some of them are widely used in the folk medicine to treat syphilis and ulcers - *J. triqueter* and depurative - *J. humilis*[5].

The ethanolic and hydroalcoholic extract from *J. triqueter* has been evaluated and presented potential antileishmanial results, with IC_{50} 29.5 μg\ mL[7-9]. Nakano et al.[10-11] and Anastasi[12] reported the isolation and structural determination of the compound julocrotine in *J. subpannosus, J. camporum* and *J. montevidensis*[13-14]. Most of the species have not been studied from the chemical and pharmacological point of view, and the activity studied had been applied only to crude fixed extracts. In this work we report the partial chemical composition of essential oil from the leaves and fruit of *J. triqueter* (Lam.) Didr. The chemical composition of the hexanic extract and its methyl ester fraction were also investigated.

MATERIALS AND METHODS

Plant material:

The botanical material was collected at Sobral – Ceará – Brazil and the vegetable material and voucher specimen were deposited at Prisco Bezerra Herbarium, UFC, under the number 27652, it was identified by L.W. Lima-Verde.

Volatile Constituent:

Fresh leaves and fruit samples (1.0 Kg), harvested at the State of Ceará, of *Julocroton triqueter* (Lam.) Didr. were subjected to hydrodistilation for 2 hours in a Clevenger-type apparatus. The isolated essential oils were dried over anhydrous sodium sulfate and, after filtration, maintained under refrigeration before analysis.

NMR Spectra:

NMR spectra were recorded on a Bruker 500 spectrometer (11.7 Tesla, 500 MHz for ^{1}H and 125 MHz for ^{13}C), in $CDCl_3$. Chemical shifts δ (in ppm) are given from internal TMS. The IR spectra were recorded on a Perkin Elmer – Model Spectrum 1000 FTIR (KBr, 4000 cm^{-1} to 400 cm^{-1}).

Gas chromatography/mass spectrometry:

Analysis of the oil was performed on HP (5890 A, II MSD) gas chromatograph, connected with MS system (HP 5971 A): dimethylsiloxane DB-5 fused silica capillary column (30 m x 0.25 mm, 0.1 m film thickness); carrier gas: helium 9 mL/min; injector temperature 250 °C; detector temperature 200 °C; column temperature 50 -180 °C at 4 °C/min, then 180-250 °C at 20 °C/min. Mass spectrometer operating conditions 70 eV ionization energy. Identification of individual components was based on their mass spectral fragmentation using two computer library MS searches (Wiley Mass Spectral Database 229), retention indices and comparison with literature data[15-18]. Relative percentage amounts were calculated from the total area under the peaks by the software of the apparatus.

Extraction and Isolation:

Dried and powdered fruits (2,8 Kg) of *J. triqueter* were extracted two times with hexane (3.5 L), followed by ethanol, at room temperature. After solvent evaporation under vacuum 14.1 of hexane and 22.0 g ethanolic extract were obtained, respectively. The ethanolic extract was then fractionated on a silica gel column with hexane, chloroform, ethyl acetate and methanol, gradient eluition, yielding 7.45, 6.90, 3.29 and 3.70 g of extract, respectively. During the process of eluition with hexane was observed the formation of a precipitate yellow, which was then washed in the cold light petroleum, yielding 80 mg of an amorphous solid white. This solid, soluble in chloroform, showed a single component on TLC and analysis by ^{1}H and ^{13}C RMN indicated the tetracosan-1-ol as constituent.

The hexane fraction (7.45 g) was subjected to saponification followed by methylation reaction[19-21], and the reaction was followed by thin-layer chromatography. The reaction mixture was purified by chromatography column and the solvent was then evaporated in a vacuum (unsaponifiable: 0.5 g; saponifiable: 5.49 g). A small portion (saponifiable) of the crude reaction was analyzed in GC-MS.

RESULTS AND DISCUSSION

The identification of the essential oil components was accomplished by comparison of their GC-MS retention indices as well as their mass spectra with corresponding data of authentic compounds of components of reference oils. To minimize the standard deviation arising from employing a sole substance as internal standard, we decided to employ a retention index, namely, Kovat's Index obtained by use of a mixture of the essential oil with eight n-alkanes as internal standards. This approach greatly improved the identification, especially for those compounds with very similar fragmentation patterns. The oils were analyzed on mono and sesquiterpenes, the main components being caryophyllene oxide (41.8%), humulene epoxide II (8.7%), *trans*-caryophyllene (8.2%), occidentalol (7.4), *α*-humulene (4.5%) and *para*-cimene (4.2%) in the fruit and *cis*-carvyl acetate (44.6 %), spathulenol (12.1%), *cis*-carveol (10.8%), *trans*-caryophyllene (6.0%), *trans*-carvyl acetate (5.5%) and *α*-humulene (2.9%) in the leaf, **Table 1**.

e-mail address: sidney@ufpi.edu.br

Table 1. Chemical composition of the essential oil, obtained from leaves and fruits of *Julocroton triqueter* (Euphorbiaceae).

Fruits of *J. triqueter*	Retention Index	*Relative Area (%)*	Leaves of *J. triqueter*	Retention Index	*Relative Area (%)*
α-terpinene	1016	2.33	*trans*-pinocarvyl acetate	1295	2.91
para-cymene	1022	4.20	linalool	1092	2.06
limonene	1028	2.43	*cis*-carveol	1225	10.77
trans-pinocarveol	1136	1.84	*trans*-carvyl acetate	1336	5.51
trans-carvyl acetate	1336	2.66	*cis*-carvyl acetate	1364	44.57
δ-elemene	1334	1.40	----------	----------	----------
β-patchoulene	1380	1.06	----------	----------	----------
β-bourbonene	1379	2.91	β-bourbonene	1379	5.16
β-elemene	1386	1.86	spathulenol	1574	12.14
E-caryophylene	1414	8.16	*E*-caryophylene	1414	6.06
α-humulene	1451	4.50	α-humulene	1451	2.91
thujopsadiene	1461	2.67	----------	----------	----------
occidentalol	1546	7.44	----------	----------	----------
caryophyllene oxide	1579	41.80	----------	----------	----------
humulene epoxide II	1603	8.67	humulene epoxide II	1603	4.27
α-muurolol	1639	2.59	----------	----------	----------

The partial analysis of the non-volatile constituents (hexane fraction) from *J. triqueter* fruit allowed the isolation of a compound, obtained in the form of an amorphous solid, soluble in chloroform, with a melting point of 75.8 - 77.3 ^{o}C (literature[22]: 77-79 ^{o}C). The ^{1}H – NMR (500 MHz, $CDCl_3$) spectrum of the compound showed signals in: 3.60 ppm (t, 2H, H-1); 1.26 ppm (s, 42H); 0,86 ppm (t, 3H, H-24). The analysis of Gated - Decoupled ^{13}C NMR (125 MHz, $CDCl_3$) and ^{13}C NMR- DEPT 135^{o} spectrum allowed us to identify signals in: 32.9 ppm (CH_2, C-2); 31.9 ppm (CH_2, C-22); 29.4 - 29.7 ppm (18 CH_2); 25.8 ppm (CH_2, C-3); 22.7 ppm (CH_2, C-23); 14.2 ppm (CH_3, C-24) and 63,1 ppm (-CH_2-O, C-1). FT-IR spectrum showed important stretching and bending vibrations : 3342 cm^{-1} (O-H stretch); 2918 cm^{-1} and 2849 cm^{-1} (sp^3 C-H absorption); 1465 cm^{-1} (methylene groups bending absorptions); 1064 cm^{-1} (C-O, stretching vibration) and 725 cm^{-1} (bending motion associated with four or more CH_2 groups in an open chain). The analysis of spectral data allowed us to identify the compound as the Tetracosan-1-ol.

The methylester analysis , obtained from the fatty material, lead to the identification of dodecanoic (0.85%), tetradecanoic (1.15%), hexadecanoic (26.92%), 9-octadecenoic (19.36%), eicosanoic (6.0 %), trieicosanoic (2.29%) and tetracosanoic acid (0.89 %) as main constituents (**Table 2)**.

Table 2. Composition of the main fatty acid detected in the fixed oil of *Julocroton triqueter* (Euphorbiaceae).

Fatty acid	Retention Time	*Relative Area (%)*
non identified	11.06	5.77
dodecanoic acid (C12:0) or lauric acid	12.51	0.85
tetradecanoic acid (C14:0) or myristic	15.41	1.15
hexadecanoic acid (C16:0) or palmitic acid	18.24	26.92
octadecanoic acid (C18:0) or stearyc acid	19.67	1.60
9-octadecenoic acid (C18:1) or oleic acid	20.43	19.36
eicosanoic acid (C20:0) or araquidic acid	23.10	6.07
trieicosanoic acid (C23:0)	25.12	2.29
tetracosanoic acid (C24:0) or lignoceric acid	27.12	0.89
Total fatty acids = 64.9%		

The results of this preliminary analysis shall be important from the pharmacological point of view, since components of the essential oil have been found to have powerful anti-inflammatory (*α*-humulene and caryophylene), anti-bacterial, anti-ulcer and anti-fungal (caryophylene and its oxide) and anti-septic (linalool) properties. It has been identified about 96% of the total constituents and in general the study showed that the essential oils present different chemical compositions in different parts of the plant, yielding ranges from 0.12 to 0.30% (w/w) on fresh weight basis. The compounds *trans*-carvyl acetate, *trans*-caryophyllene, *α*-humulene and humulene epoxide were found in essential oils from both leaves and fruits. Unfortunately, there is no other study on *Julocroton* oil in the literature for comparison purposes and because there is no available data on the biological activity, variability of the composition of essential oil content with regard to season, climate and age, the effects of these variables will be currently studied in our laboratory.

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