


Contribution to the chemical study of *Chrysobalanus icaco* leaves

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ABSTRACT

Chrysobalanus icaco (Chrysobalanaceae), is a shrub in Brazil, occurs throughout the coast and in the Amazon biome, known as abajurú, abajerú, bajerú, guajurú, among other popular names. In folk medicine, it is mainly used to control blood glucose in diabetics, dysentery and leukorrhea. There are studies in the scientific literature that confirm its antidiabetic effects. From a chemical point of view, the species is characterized as a bioproducer of terpene and phenolic compounds. The objective of this work is to contribute to the chemical studies of the species *C. icaco*, considering its relevant pharmacological activities. In this work we present the isolation and characterization of two triterpenes and three flavonoids, in addition to the product obtained by acetylation reaction of an ethyl acetate fraction identified as $2\alpha, 3\beta$ -diacetoxy- 6β -hydroxy-olean-12-en-28-oate of [β -D-triacetoxy-glycopyranosyl (2 \rightarrow 1) - β - triacetoxy-xylopyranosyl]. The structural determination of the substances was made through the analysis of 1D and 2D nuclear magnetic resonance spectra and HR-ESI-MS mass spectrometry and comparison with values recorded in the literature.

Keywords: *Chrysobalanus icaco*, Chrysobalanaceae, Triterpenos, Flavonoides.

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INTRODUCTION

The family Chrysobalanaceae comprises 17 genera and about 525 species represented by trees and shrubs widely distributed in tropical and subtropical areas [1]. *Chrysobalanus* is a small genus of this family, composed of the species *Chrysobalanus icaco*, *Chrysobalanus cuspidatus*, and *Chrysobalanus venezuelanus*, widely distributed in tropical America and tropical Africa [2]. The species *C. icaco*, in Brazil, occurs throughout the coast and in the Amazon biome it is known as abajurú, abajerú, bajarú, guajurú, among other popular names. It has edible fruits and various parts of the plant are used in folk medicine. Roots, bark and leaves are astringent and employed against dysentery, leukorrhea, kidney stones [3]. Aqueous extracts of its leaves are commonly used for blood glucose control in diabetics [4]. There are also studies in the scientific literature that, in addition to confirming its antidiabetic effects, report inhibitory effects on the HIV-1 virus, anti-inflammatory and antinociceptive activities, leishmanicidal, antioxidant, genotoxic, antimicrobial, antiangiogenic, anti-cancer and anti-cancer resistant to multi-drugs, prevention against obesity, decreases doxorubicin-induced DNA damage [5-10]. From a chemical point of view, the species that present reports in the literature are *C. icaco*, followed by *C. venezuelanus*, giving the genus *Chrysobalanus* a rich source of terpenes and phenolic compounds [2,5,7]. Among the terpenes, kaurane-type diterpenes have been recorded [10]. and triterpenes mainly with oleanan and ursan skeletons [2.5.6]. Of the phenolic compounds, the flavonoids myricetin, quercetin and their O-glycosylated derivatives stand out, as well as catecholic tannins [2,4,5] and anthocyanins [7]. Flavonoids, particularly myricetin and its glycosides, have been used as chemotaxonomic markers in the family *Chrysobalanaceae* [2,4]. In this work we present the isolation and characterization of two olean skeleton triterpenes, the flavonoids quercetin, kaempferol and 3-O- β -D-glycopyranosyl myricetin, as well as a product obtained by acetylation reaction of a fraction of the ethyl acetate partition of the methanol extract of *C. icaco* leaves identified as 2 α , 3 β -diacetoxy-6 β -hydroxy-olean- $[\beta$ -D-triacetoxy-glycopyranosyl(2 \rightarrow 1) β -D-triacetoxy-pyranosyl] 12-en-28-oate. The structural determination of the substances was made through the analysis of 1D and 2D nuclear magnetic resonance spectra and HR-ESI-MS mass spectrometry and comparison with values recorded in the literature. The presence of these flavonoids may be responsible for its anti-oxidant and anti-inflammatory activity, and may attribute the hypoglycemic activity to myricetin [4].

EXPERIMENTAL PART

INSTRUMENTATION

The NMR¹H and NMR¹³C spectra (1D and 2D) were obtained in Bruker Avance II 400 and Bruker Avance III 500 spectrometer operating at the hydrogen frequencies 400MHz and 500 MHz and at the carbon-13 frequencies at 100 MHz and 125 MHz from the analytical center of the Institute



of Chemistry of the Federal Rural University of Rio de Janeiro. The solvents used in the dissolution of the samples were deuterated chloroform, methanol and pyridine. The high-resolution mass spectra were obtained in a MICROTOF-Bruker Daltonics spectrometer equipped with an electrospray ionization source from the Analytical Instrumentation Center of the University of São Paulo (Laboratory of Mass Spectrometry).

MATERIAL VEGETAL

Leaves of *C. icaco* were collected in the municipality of Rio de Janeiro, Brazil and identified by Dr. Rosa Fuks from Jardim Botânico/RJ. An exsicata of the species was deposited in the Herbarium of the National Museum (R195941) of UFRJ. SIsGen Registration: AEADE65.

EXTRACTION AND ISOLATION OF CHEMICAL CONSTITUENTS

Dried and ground leaves (2,500g) were subjected to the maceration process at room temperature using methanol as solvent (5L). Part of the 3-liter methanolic extract was subjected to solvent removal through a rotary evaporator under reduced pressure, generating (220g) of the dry extract. From this, a 90g aliquot was solubilized in methanol/H₂O solution (8:2) and subjected to liquid/liquid partitioning with the solvents hexane, ethyl acetate and butanol in increasing order of polarity to provide four fractions. The ethyl acetate fraction, after removal of the solvent by distillation under reduced pressure, provided 50.0 g of residue, of which 5.0 g was subjected to a silica gel chromatographic column (70-230 *mesh*) eluted with mixtures of Hex:AcOEt:MeOH solvents in a gradient of increasing polarities, originating 20 fractions of 200 mL. These fractions were analyzed by thin layer chromatography and regrouped according to their chromatographic profile. The group of fractions 9-12 (170.0 mg) was rechromatographed in a silica gel column using the CHCl₃/MeOH mixture (9:1) as eluent, producing triterpenes **2** (20.0mg) **and 3** (32.0mg). Sub-fractions 14-16 (220mg) were pooled and submitted to a Sephadex-LH 20 column eluted with 100% methanol generating 10 fractions. The CCDA analyses revealed with Liebermann-Burchard, showed a violet coloration, indicating the presence of triterpenes and that fractions 3 to 7 were similar despite being impure, so they were gathered (40.0mg) and then subjected to an acetylation reaction using acetic anhydride and pyridine (1:1) and left at room temperature with agitation for 24 hours. The product of this reaction, after purification in a small silica gel filter column and analysis of NMR spectra, allowed to be identified as substance **1** (19.0 mg). 20 g of the butanolic fraction was submitted to a chromatographic process in an eluted silica gel column with a binary mixture of the solvents ethyl acetate and methanol, in a gradient of increasing polarities. 27 fractions of 300 ml were obtained. Fractions 1-6 after development with sulfuric vanillin were presented as a mixture of phenolic compounds. These were gathered and submitted to a new chromatographic process in a

Sephadex LH-20 column and 8 new subfractions were obtained; Of these, subfraction 2 was purified by filtration in silica to provide flavonoids **4** (15.0 mg) and **5** (11.0 mg). Fractions 5-6 of the latter column were gathered and rechromatographed in Sephadex LH-20, leading to the isolation of flavonoid **6** (8.0 mg).

RESULTS AND DISCUSSION

The phytochemical study of the methanol extract of *C. icaco leaves* led to the isolation and identification of two triterpene acids (**2** and **3**), flavonoids **4**, **5** and **6**, in addition to the substance obtained from the acetylation reaction of an ethyl acetate fraction, substance **1**. From the ethyl acetate fraction from the liquid/liquid partition of the methanol extract of leaves, the following were isolated: triterpenic acids, 2 α -3 β -6 β -trihydroxy-olean 12-en-28-oic (**2**) and 2 α -3 β -6 β -23-tetrahydroxy-olean 12-en-28-oic (**3**) and obtained the substance of the acetylation reaction, 2 α , 3 β -diacetoxy-6 β -hydroxy-olean-[\beta-D-triacetoxy-glycopyranosyl 12-en-28-oate (2 \rightarrow 1)-\beta-triacetoxy-xylopyranosyl] (**1**); From the butanolic fraction from the liquid/liquid partition of the methanol extract of the leaves, the flavonoids quercetin (**4**), kaempferol (**5**) and 3-O-\beta-glycopyranosyl-myricetin (**6**) were isolated. The structures of the isolated compounds were determined through the analysis of the ¹H and ¹³C NMR spectra, DEPT, HMBC, HSQC, COSY and by comparison with data described in the literature. Substance **1** was obtained as a white solid. The high-resolution mass spectrum of substance **1** showed the peak at *m/z* 1141.5338 ([M+Na⁺], C₅₇H₈₂NaO₂₂, calculated: 1141.4965) compatible with the molecular formula C₅₇H₈₂O₂₂. In the ¹H NMR spectrum, seven singlets δ H 0.93, 0.94, 0.98, 1.01, 1.13, 1.34, 1.49 corresponding to seven methyl groups, a broad simplete at δ H 5.36 characteristic of olefin hydrogen, was observed. In the NMR spectrum, ¹³C in the olefins region shows signals in δ C 122.56 (CH) and δ C 142.46 (C). These ¹H and ¹³C NMR data allow us to propose the presence of a triterpene structure with an Olean-12-en skeleton [11]. The presence in the ¹H NMR of the broad doublet at δ H 2.86 (J= 11Hz) compatible with H -18 [11] also corroborates the Olean skeleton [11]. The δ H 5.20 (H -2 dt, J=10; 10; 4.0Hz) and δ H 4.71 (H-3 d, J=10 Hz) present in the ¹H NMR spectrum and correlations observed with acyl groups (δ C 170.6 and 171.0 respectively) through HMBC contour map (Table 1) made it possible to recognize the presence of a 2 α , 3 β di-O-acyl system [12]. The presence of glycosidic units was also detected through the analysis of the HMBC contour map, where ³J_{CH} correlations were observed between the δ H 5.45 anomeric methinic hydrogen (H-1', d, J= 8Hz) and δ H 4.71 anomeric methinic hydrogen (H-1'', d, J=8.0 Hz) with the carbons δ C 91.5 (C-1') and δ C 100.7 (C-1''), respectively, allowing the presence of two sugar units to be proposed and according to the coupling constants of their Anomeric hydrogens are in β configuration. In the ¹³C/NMR spectrum (DEPT) eight signals of quaternary carbons between δ C 169.70 – 171.00 attributed to acyl groups incorporated into the molecule were

observed, which had their locations established through analysis of the HMBC contour map. Still analyzing the $^{13}\text{C}/\text{NMR}$ spectrum (DEPET), the presence of carbinolic methinic carbons compatible with the presence of one glucose unit and one xylose unit characterized mainly by δC 62.5 (CH_2 , C-5'') are observed. The determination of the sequence of hydrogens relative to each unit of monosaccharides was performed through homonuclear correlations in the COSY spectrum: Glucose [δH 5.46(H-1') \leftrightarrow 3.94(H-2') \leftrightarrow 5.28 (H-3') \leftrightarrow 5.0 (H-4') \leftrightarrow 3.78 (H-5') \leftrightarrow 4.30 (H-6a') \leftrightarrow 4.06 (H-6b')]; Xylose [δH 4.60 (J=8.0 Hz, H-1'') \leftrightarrow 4.85 (H-2'') \leftrightarrow 5.15(H-3'') \leftrightarrow 4.93 (H-4'') \leftrightarrow 4.12 (H-5a'') \leftrightarrow 3.31 (H-5''b)]. The union between the monosaccharide units was determined by the long-distance correlation $^3\text{J}_{\text{CH}}$ between δH 3.94 (H-2') of glucose and δc 100.7 (C-1'') of xylose. And the heteroside position was determined by correlating δH 5.45 (H-1') of glucose and δC 175.6 (C-28) of aglycone observed in the HMBC contour map. The presence of carbinolic methinic carbon at δC 67.75 (C-6), not correlated to the acyl group and presenting $^1\text{J}_{\text{CH}}$ with a broad simplet, δH 4.55, is compatible with the presence of a hydroxyl group at the 6β position (axial), presenting a 1.3 diaxial interaction with methyl group 10β δC 17.78 (CH_3 -25), making it aglycone similar to 6β hydroxymaslinic acid [13-15]. The data obtained allowed the characterization of substance **1** as being $2\alpha,3\beta$ -diacetoxy- 6β -hydroxy-olean-12-en-28-oate of [β -D-triacetoxy-glycopyranosyl (2 \rightarrow 1) - β - triacetoxy-xylopyranosyl]. The structures of the known compounds **2-6** were defined by interpreting the NMR spectra ^1H and ^{13}C and comparing them with the literature for 2α - 3β - 6β -trihydroxy-olean 12-en-28-oico2 [13], 2α - 3β - 6β -23-tetrahydroxy-olean 12-en-28-oico3 [16], quercetin **4** [17], Kaempferol **5** [18], 3-O- β -glycopyranosyl-myricetin **6** [19].

Figure 1. Structures of substances isolated from the methanol extract of *C. Icaico* leaves.

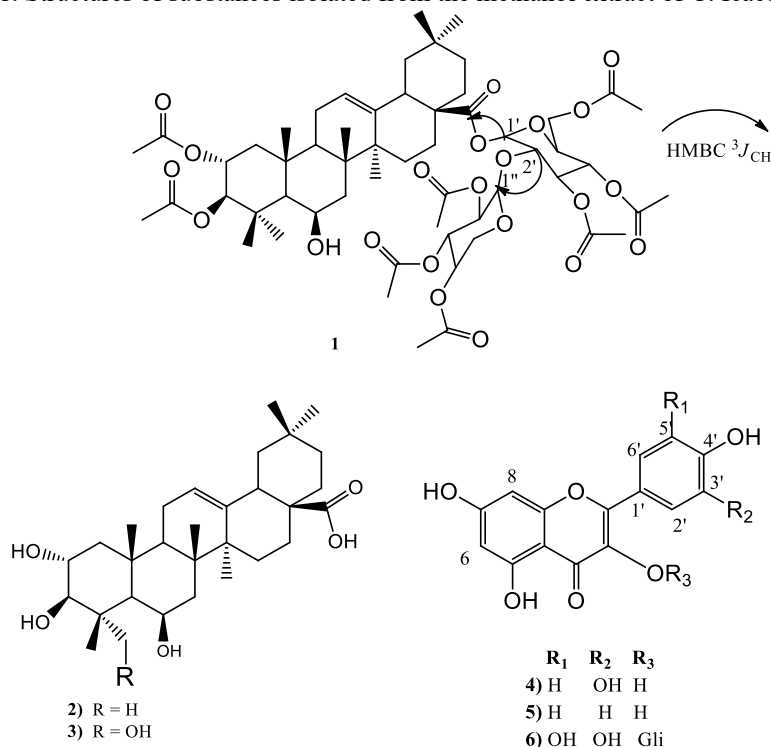


Table 1: ¹H (400 MHz) and ¹³C (100 MHz) NMR data, CDCl₃, of substance 1.

| C | HSQC | dH(mult, <i>J</i> em Hz) | HMBC | | ¹ Hx ¹ H-COSY |
|-----|--------|---|---------------|------------------------|-------------------------------------|
| | CDs | | Day 2 | Day 3 | |
| 1 | 46,65 | 1,99 sl | C-2; C-10 | C-5 | |
| 2 | 70,22 | 5,20 (dt,10,0; 10,0; 4,0) | | AcO(170,63) | H-3 |
| 3 | 80,90 | 4.71 (d, 10) | C-2; C-4 | C-24; C-23; AcO(171,0) | |
| 4 | 40,67 | | | | |
| 5 | 55,37 | 0,9 s | | | |
| 6 | 67,75 | 4,55 sl | | | H-5 |
| 7 | 39,99 | | | | |
| 8 | 38,58 | | | | |
| 9 | 47,89 | | | | |
| 10 | 37,77 | | | | |
| 11 | 22,13 | | | | |
| 12 | 122,56 | 5,36 sl | | C-9; C-14 | |
| 13 | 142,69 | | | | |
| 14 | 42,16 | | | | |
| 15 | 28,74 | | | | |
| 16 | 23,40 | | | | |
| 17 | 45,53 | | | | |
| 18 | 41,19 | 2,86 (d, 11,0) | | C-12 | |
| 19 | 45,89 | | | | |
| 20 | 30,64 | | | | |
| 21 | 33,73 | | | | |
| 22 | 31,42 | | | | |
| 23 | 28,20 | 0,98 s | | C-24 ; C- 4; C-5; C-3 | |
| 24 | 19,16 | 1,34 s | | C-23 ; C-4 ; C-5; C-3 | |
| 25 | 17,78 | 1,49 s | C-10 | C-1; C-5 | |
| 26 | 17,93 | 1,01 s | C-8 | C-7 ; C-14 ; C-9 | |
| 27 | 25,95 | 1,13 s | C-14 | C-15; C-8 | |
| 28 | 175,63 | COOH | | | |
| 29 | 33,10 | 0,93 s | | C-30 / C-21 | |
| 30 | 23,58 | 0,94 s | | C-23 / C-21 | |
| 1' | 91,58 | 5,45 (d, 8,0) | | C-28 | H-2' |
| 2' | 74,47 | 3,94 t | C-3'; C-1' | C-1'' | H-3' |
| 3' | 74,94 | 5,26 t | C-4', C-2' | AcO | H-4' |
| 4' | 68,01 | 5,0 t | C-5' | AcO | H-5' |
| 5' | 72,54 | 3,8dl | C-4' | | H-6'a |
| 6' | 61,48 | 4,30 (dd, 4,3; 12,4 e 4,06 (m) | | AcO | H-6b' |
| 1'' | 100,75 | 4,71 (d, 8,0) | | C-3'' | H-2'' |
| 2'' | 71,52 | 4,88 t | C-1'' , C-3'' | AcO | H-3'' |
| 3'' | 72,57 | 5,13 t | C-2'' | AcO | H-4'' |
| 4'' | 68,09 | 4,93m | | AcO | H-5'' |
| 5'' | 62,54 | 4,12 (dd, 12,0 e 5,6) / 3,31(dd, 12,0 e 4,0 | | C-1'' | H-5''a/H-5''b |

2 α , 3 β -diacetoxy-6 β -hydroxy-olean-12-en-28-oate of [β -D-triacetoxi-glucopyranosyl (2 \rightarrow 1) – β - triacetoxi-xylopiranosyl] (1). White solid, NMR ¹³C (100 MHz, CDCl₃) δ (ppm) as given in Stem 1.

2 α -3 β -6 β -trihydroxy-olean-12-en-28-oic acid (2). White solid, NMR RMN ¹³C (100 MHz, C₅D₅N) δ (ppm) 46,5 (C-1), 68,6(C-2), 83,8(C-3), 41,0(C-4), 56,3(C-5), 67,3(C-6), 42,6(C-7), 39,0(C-8), 48,5(C-9), 38,1(C-10), 23,6(C-11), 122,2(C12), 144,4(C13), 42,0(C-14), 28,1(C-15), 23,6(C-16), 46,7(C-17), 42,5(C-18), 49,8(C-19), 30,8(C-20), 34,2(C-21), 33,2(C- 22), 28,9(C-23),

19,0(C-24), 18,5(C-25), 18,2(C-26), 26,4(C-27), 181,0(C-28), 33,2(C-29), 23,6(C-30). The chemical displacements of the carbon atoms of 2 were attributed by the analysis of the 2D spectra of heteronuclear correlation HSQC, HMBC and comparison with data described in the literature [13].

2 α -3 β -6 β -23-tetrahydroxy-olean-12-en-28-oic acid (3). White Solid RMN ^{13}C (125 MHz $\text{C}_5\text{H}_5\text{N}$ δ (ppm) 49,7 (C-1), 68,8(C-2), 77,9(C-3), 44,3(C-4), 48,5(C-5), 67,3(C-6), 40,7(C-7), 39,0(C-8), 48,5(C-9), 37,9(C-10), 23,5(C-11), 122,3(C12), 144,7(C13), 42,5(C-14), 28,4(C-15), 23,8(C-16), 46,5(C-17), 41,9(C-18), 46,3(C-19), 30,7(C-20), 34,0(C-21), 33,0(C-22), 65,7(C-23), 15,7(C-24), 18,7(C-25), 18,4(C-26), 25,9(C-27), 180,0(C-28), 33,0(C-29), 23,5(C-30). The chemical displacements of carbon atoms of 3 were attributed by the analysis of the heteronuclear correlation 2D spectra HSQC, HMBC and comparison with data described in the literature [16].

Quercetin (4). White/yellowish solid NMR ^{13}C (125 MHz CD_3OD δ (ppm): 147,3 (C-2); 135,8 (C-3); 175,9 (C-4); 161,1 (C-5); 97,8 (C-6); 164,1 (C-7); 92,9 (C-8); 156,8 (C-9); 103,0 (C-10); 122,7 (C-1'); 114,5 (C-2'); 144,8 (C-3'); 146,5 (C-4'); 114,7 (C-5'); 120,4 (C-6'). The chemical displacements of carbon atoms of 4 were attributed by the analysis of the 2D heteronuclear correlation spectra HSQC and HMBC and comparison with data described in the literature [17].

Kaempferol-(5) yellowish solid: RMN ^1H (500 MHz CD_3OD δ (ppm): 8.1 2H ($J=8,8$ Hz; H-2' e H-6'); 6,92 ($J=8,8$ Hz H-3' e H-5'); 6,42 (H-6); 6,20 (H-8). RMN ^{13}C (125 MHz CD_3OD δ (ppm): 146 (C-2); 97,8 (C-6); 93,0 (C-7); 103 (C-9); 121, (C-1'); 129,2 (C-2'/6'); 114,8(3'/5'); 159 (C-4').. The chemical displacements of the carbon atoms of 5 were attributed by the analysis of the 2D heteronuclear correlation spectra HSQC and HMBC and comparison with data described in the literature [18].

Myricetin-3-O- β -D-glycopyranosyl (6) RMN ^{13}C (125 MHz CD_3OD δ (ppm): 157,0 (C-2); 134,2 (C-3); 178,2 (C-4); 162,0 (C-5); 98,4 (C-6); 164,0 (C-7); 93,2 (C-8); 156,0 (C-9); 104,0 (C-10); 120,0 (C-1'); 108,4 (C-2'); 146,0 (C-3'); 134,2 (C-4'); 146,0 (C-5'); 108,4 (C-6'); 103,1 (C-1''); 73,8 (C-2''); 76,1 (C-3''); 69,6 (C-4''); 76,1(C-5''); 65,8(C-6''). The chemical displacements of the carbon atoms of 6 were attributed by the analysis of the 2D spectra of heteronuclear correlation HSQC and HMBC and comparison with data described in the literature [19].

FINAL CONSIDERATIONS

The chemical study of *Chrysobalanus icaco* led to the isolation of triterpenic acids: 2 α -3 β -6 β -trihydroxy-olean-12-en-28-oico 2; 2 α -3 β -6 β -23-tetrahydroxy-olean-12-en-28-oico 3; flavonoids: quercetin 4; kaempferol 5, myricetin 3-O- β -glycopyranosyl 6, in addition to obtaining and characterizing 2 α ,3 β -diacetoxy-6 β -hydroxy-olean-12-en-28-oate of [β -D-triaacetoxy-glycopyranosyl(2 \rightarrow 1)- β -trioacetoxy-xylopyranosyl] substance 1, prepared by acetylation reaction of an ethyl acetate fraction. The proposed structure for substance 1 is compatible with an acetylated



triterpene saponin. The obtaining of this substance, more nonpolar, by means of a simple acetylation process (acetic anhydride/pyridine stirring for 24 hours) suggests the presence of triterpene saponins among the chemical constituents of the species. The complexity of the structure and the high degree of polarity of this class of metabolites make it difficult to isolate it in natura by traditional chromatographic methods. Saponins can often undergo hydrolysis reactions in the plant extract or in the column, thus allowing the isolation of only aglycones, such as triterpenic acids 2 and 3. The work contributes to the chemical study of the species *Chrysobalanus icaco*, presenting new constituents of its metabolites, confirming that myricetin derivatives are chemical markers of the Chrysobalanaceae family and that it is a bioproducer of terpenoidic and flavonoid compounds.

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