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PHYTOCHEMICAL STUDY AND ANTIMICROBIAL ACTIVITY OF *Chrysobalanus icaco*

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Chrysobalanus icaco L. belongs to the Chrysobalanaceae family. The Chrysobalanaceae encompasses 17 genera and about 450 species represented by trees and shrubs growing in tropical and subtropical lowlands. In El Salvador, Trinidad, and Brazil, an infusion or decoction of *C. icaco* fruits, leaves, bark, or roots, cures chronic diarrhea, dysentery, hemorrhages, and leucorrhea [1]. *C. icaco* leaf infusion is also used popularly in Brazil as a diuretic and as a hypoglycemic. These pharmacological activities have been experimentally proved [2, 3]. The organic extract of *C. icaco* showed an *in vitro* inhibitory effect on HIV l infection [4]. The methanol extract from leaves of *C. icaco* reduces the formation of new blood vessels in chicken chorioallantoic membrane [5]. The pomolic acid isolated from *C. icaco* inhibited the growth and induced the apoptosis of K562; it also inhibited the proliferation of Lucena 1, a vincristine-resistant cell (MDR) [6]. The aqueous extract prepared from leaves of *C. icaco* shows a potential genotoxic effect and antioxidant action [7]. The chemical composition of Chrysobalanaceae species includes flavonoids, terpenoids (triterpenes and diterpenes), steroids, and tannins [8, 9]. *C. icaco* has been poorly investigated from the chemical point of view. Gustafson and et al. [4] isolated two diterpenes from this species that showed activity in the anti-HIV screen.

C. icaco was collected in 1997 in Rio de Janeiro state and identified by Dr. Rosa Fuks from the Botanical Garden of Rio de Janeiro. A voucher specimen has been deposited at Museu Nacional Herbarium of UFRJ (R195.941).

Dried and powdered leaves of *C. icaco* were extracted by maceration at room temperature successively with hexane and methanol. The methanol crude extract was partitioned with hexane, CH_2Cl_2, EtOAc, and BuOH, successively. The crude hexane extract (8 g) was chromatographed on silica gel column and eluted using binary mixtures of hexane, EtOAc, and MeOH to afford stigmasterol, sitosterol, and campesterol. Purification of the CH_2Cl_2 fraction (70 g) by silica gel column yielded pomolic acid. A portion of the BuOH fraction (230 mg) was further purified by preparative reverse-phase HPLC separation to give 7-*O*-methylkaempferol.

The identification of these compounds was supported by GC/MS, ^1H, and ^{13}C NMR, melting points, and optical rotations. All compounds were identified by comparison with reported physical and spectral data [10]. The pomolic acid and 7-*O*-methylkaempferol were isolated from *C. icaco* for the first time.

The microbial panel included laboratory control strains from the American Type Culture Collection (Rockville, MD, USA): *Staphylococcus aureus* (25923), *Pseudomonas aeruginosa* (15422), *Streptococcus pyogenes* (75194), and *Escherichia coli* (25922), and the yeasts *Candida albicans* (10231). The dried plant crude extracts, fractions (50 mg/mL), and isolated compounds (1 mg/mL) were dissolved in the same solvents of the extracts and fractions from their origin. Antimicrobial tests were carried out by the disc-diffusion method [11]. In order to evaluate the sensivity of the test organisms, the following antibiotics (30 μg) were tested: tetracycline, chloramphenicol, penicillin, gentamicin, and cephalotin (CHEMCO). Sterile discs impregnated with solvents were used as negative controls. The experiments were performed in triplicate and repeated three times. For 7-*O*-methylkaempferol, the minimal inhibitory concentration (MIC) was determined by the agar dilution method [12].

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The agar diffusion assay showed that the MeOH crude extract, hexane, CH_2Cl_2, and BuOH fractions presented antibacterial activity against *S. aureus* and *S. pyogenes*, 15.0 ± 1.7, 9.3 ± 0.6, 10.6 ± 0.6, 16 ± 5.2 and 18.3 ± 6.3, 7.0 ± 1.7, 10.0 ± 1.0, 18.3 ± 2.9, respectively. The hexane extract and EtOAc fraction were not active against all the microorganisms in the concentrations evaluated. The MIC values for 7-*O*-methylkaempferol were about 60 μg/mL for *S. aureus* and *S. pyogenes.* All crude extracts, fractions, and isolated substances were not active against *C. albicans* in the concentrations evaluated. The present results support the ethnopharmacological use of this species as anti-infectious agent. 7-*O*-Methylkaempferol was shown to present *in vitro* antimicrobial activity. This is the first report on the chemistry and antimicrobial activity of *C. icaco*.

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