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Research Article

Pentacyclic triterpenoids from the leaves of *Cecropia longipes*

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Abstract

The genus *Cecropia* (Urticaceae) comprises 61 tree species primarily found in tropical rainforests from Mexico to South America, including Argentina, Brazil, Paraguay, and Panama. These species are under-researched, with most studies focusing on phenolic derivatives from leaf extracts. Traditionally, *Cecropia* species are used to treat ailments such as asthma, bronchitis, and diabetes. This study focuses on the underexplored species *C. longipes* from Panama, aiming to analyze triterpene derivatives in its leaves. Through a series of chromatographic separations and purifications, three pentacyclic triterpenoids were isolated. Using 1D and 2D NMR experiments and HRESI-MS analysis, the following compounds were identified: 19α hydroxyasiatic acid, 1α,3β,23-trihydroxyolean-12-en-28-oic acid, and rosamultic acid. These compounds were isolated for the first time from *C. longipes* and the genus *Cecropia*. Genotoxicity tests using *S. cerevisiae* revealed that two compounds exhibited genotoxic activity at all concentrations. For 19α hydroxyasiatic acid, genotoxic potential was observed at higher concentrations, unrelated to oxidative stress, suggesting other mechanisms.

Keywords

Cecropia longipes, isolation, pentacyclic triterpenoids, genotoxicity

Introduction

The genus *Cecropia* Loefl. (*Urticaceae*) is widely distributed throughout the tropical and subtropical regions from Mexico to South America, including Argentina, Brazil, Paraguay, and Panama. It is represented mainly by trees and comprises 61 species. *Cecropia* trees typically range from 5–25 m in height with a sectioned stem and upright, hollow, and broad leaves with unique textures forming umbrella-like shapes. Many of these species have stinging trichomes (plant hairs) all over the stems and leaves that cause a painful rash when in contact with the skin (Berg and Franco Rosselli 2005). Ethnobotanical approaches of *Cecropia* species are usually related to traditional uses in treating conditions like asthma, bronchitis, diabetes, and chronic kidney diseases and are broadly known for their anti-inflammatory, antioxidant, hypoglycemic, hypotensive, sedative, and antidepressant effects. Furthermore, infusion of *C. obtusifolia* is used for the treatment of nerve conditions, such as fever reducer (antipyretic), cardiac conditions, liver and pulmonary diseases, flu, wounds, scorpion and ant bites, bone fractures, rheumatoid arthritis, and wart remov-

al, among many others. While the genus has numerous ethnopharmacological applications, 14 species are specifically noted for their significant use (Cadena-Zamudio et al. 2018). Commonly reported metabolites from the genus *Cecropia* are terpenoids, steroids, and phenolic compounds such as proanthocyanidins and flavonoids. The terpenes, classified into mono, di, tri, tetra, and sesquiterpenes depending on the isoprene unit, are a diverse class of biologically active substances found in plants but also in some animals. The ones in *Cecropia* are generally pentacyclic terpenoids. Euscaphic acid, tormentic acid, and 2α- and 3β-acetyl tormentic acid have been isolated from the bark of *C. lyratiloba*. (Rocha et al. 2007a). The extraction process, including purification with column chromatography and preparative TLC, led to isolating β-sitosterol, 2-*O*-acetyl-tormentic acid, 2-*O*-acetyl euscaphic acid, and tormentic acid from *C. catharinensis*. The extended purification also issued other compounds such as pomolic and 2,3,23,-trihydroxy-olean-12-en-28-oic acids. Pomolic acid was derived from the dry and powdered aerial parts of *C. pachystachya*, traditionally used for diabetes in folk medicine, and was extracted using dichloro-methane in a Soxhlet apparatus and subsequently isolated via chromatographic processes and confirmed by NMR (Machado et al. 2008). Furthermore, serjanic and spergulagenic acids were isolated from the roots of *C. telenitida* after using various purification and chromatographic methods. In the same research, yarumic acid was found in lower amounts, and 20-hydroxy-ursolic acid and goreishic acid I were detected in trace amounts. The structures of those compounds were confirmed by various spectral analyses (Montoya Peláez et al. 2013). The following triterpenes were isolated from fresh plant specimens of *C. obtusa and C. palmata*: α-amirin, β-amirin, lupeol, maslinic, and oleanolic acid. Additionally, erythrodiol was found in *C. palmata*. The methods used for the extraction include ultrasound-assisted extraction, where ethyl acetate showed to be a more effective solvent than chloroform for most compounds (Schmidt et al. 2018). Through semi-preparative HPLC analysis of several *Cecropia* species (*C. peltata*, *C. obtusifolia*, *C. hispidissima*, *and C. insignis*), pentacyclic triterpenes as euscaphic acid 28-*O*-glucoside, triterpenoid isomers of saponin-*O*-hexoside, and tormentic acid 28-*O*-glucoside (tormentoside) were isolated, as well as an inseparable mixture of niga-ichigoside F2 and buergericic acid 28-*O*-glucoside (Rivera-Mondragón et al. 2019). Pentacyclic terpens (isoyarumic acid, tormentic acid, arjunolic acid, and hederagenic acid) were extracted from the roots of *C. telenitida*, found mainly in the Colombian region, with the help of a semi-pilot extraction and preparative HPLC purification process. The whole process took 14 days and was conducted at room temperature and under constant stirring (Gutiérrez et al. 2021). The antioxidant capacity of the genus *Cecropia* has been widely studied. Aqueous extracts of *C. hololeuca* cortex and methanolic, hydroethanolic, and ethanolic extracts of *C. pachystachya* leaves showed more than 50% free radical scavenging capacity when tested with different antioxidant models such as phosphomolybdenum, 1,1-diphenyl-2-picrylhydrazine, carotene/linoleic acid bleaching agents, and thiobarbituric acid reactive agents. *C. obtusa* leaf extract prevented the formation of UV-induced pro-inflammatory cytokines IL-1β and IL-6 in HaCaT keratinocytes, which results in protective effects on keratinocytes from UVA-induced damage (Alves et al. 2019). Gels containing 2% and 5% EtOAc extract of *C. pachystachya* leaves potentiated the healing process in rats, leading to lower rates of neovascularization and cellularity, better tissue repair, and younger and more homogeneous tissue compared with the control. Like *C. pachystachya* extract, aqueous and ethanolic extracts of *C. peltata* leaves significantly reduced wound areas in animals and showed clean wounds with healthy granulation tissue. Increased protein, hydroxyproline, and hexosamine content, as well as better collagen uptake, were observed in all treatment groups compared to the respective controls (Duque et al. 2016). A standardized hydroalcoholic leaf extract of *C. glaziovii* has been shown to improve glucose tolerance in diabetic rats; aqueous and butanol extracts of *C. obtusifolia* significantly reduced blood glucose levels in diabetic rats, diabetic mice, and hyperglycemic rabbits, and its methanolic extract reduced plasma glucose in healthy mice. The hypoglycemic effect of the aqueous extract of *C. obtusifolia* showed hypoglycemic effects similar to 3 mg/kg glibenclamide and was not accompanied by increased plasma insulin levels, suggesting that this effect is not related to pancreatic beta cell stimulation (Arend et al. 2015). Aqueous extract of *C. glaziovii* leaves reduced pro-inflammatory cytokines, cellular infiltrates, myeloperoxidase activity, nitrite/nitrate concentration, lactate dehydrogenase activity, and total protein levels with a concomitant reduction in all parameters associated with carrageenan-induced oxidative damage. Chlorogenic acid, isoorientin, and isovitexin have been reported as the main compounds of this active extract (Müller et al. 2016). Aqueous extract of C. *hololeuca* bark reduced mouse paw edema and decreased production of nitric oxide, TNF-α, and IL-1β on murine macrophage J774A.1 cells (Machado et al. 2021). Nonpolar extracts of *C. pachystachya* leaves, such as hexane and dichloromethane, and the main compounds β-sitosterol and pomolic acid, reduced the inflammatory response in carrageenan-induced mouse paw edema. Furthermore, pomolic acid reduces in vivo production of IL-1β by affecting neutrophil viability through apoptosis. Trans-phytol, α-amyrin, and ursolic acid, also identified in the dichloromethane extract, also showed anti-inflammatory effects (Hoscheid et al. 2020). The flavonoid-rich butanol fraction of *C. peltata* showed anticonvulsant, anxiolytic, and sedative effects in mice at doses of 80 mg/kg. The mentioned effects can be observed to a more pronounced degree when ethanol, water, and hexane fractions are used (Chávez et al. 2013). The aqueous extract of *C. glaziovii* leaves and its butanol fraction produced an antidepressant effect. The results suggest that this effect may be due to the blockade in the uptake of monoamines, mainly [3*H*]-norepinephrine (Rocha et al. 2007). In addition to this, an antidepressant effect was observed with oral administration of *C*-glycoside-enriched flavonoid fraction and aqueous extract obtained from the leaves of *C. pachystachya*. These extracts prevented both behavioral

(hyperlocomotion) and pro-oxidant effects (increased lipid peroxidation and protein carbonyl formation and decreased total thiol content) of ketamine in a rat model of mania. These effects are believed to be mediated by chlorogenic acid, orientin, isoorientin, isovitexin, and isoquercitrin occurring in both extracts. The sedative effect produced by the aqueous extract of *C. pachystachya* (180–600 mg/kg) may be comparable to diazepam at a dose of 10 mg/kg. *C. pachystachia* has also been investigated for antinociceptive effects. The methanolic extract of its leaves was effective, dose-dependently, against acetic acid-induced pain in the second phase of the formalin test (Gazal et al. 2014).

Materials and methods

Plant material

In the present study were used leaves from *Cecropia longipes* Loefl., collected in Panamá Oeste (Arraiján) province, 8°55'59"N, 79°44'20"W, November 2021. The taxonomic classification was carried out by the botanist Orlando O. Ortiz and deposited at the Herbarium of the University of Panama, voucher № 4004.

General experimental procedures

All solvents, including EtOAc, MeOH, $CHCL_3$, CH_2CL_2, and C_6D_5N, were sourced from Fischer Chemicals (Loughborough, UK). Water for assays was collected from a dispenser via a Milli-Q system from Millipore (Bedford, MA, USA) and filtered through a 0.22 µM membrane filter before use. Analytical thin layer chromatography plates used were TLC Silica gel 60 F_{254} (20 × 20). Chromatographic plates were observed after development with the appropriate reagent. The anisaldehyde-sulfuric acid reagent was prepared by dissolving 0.500 mL of anisaldehyde in 10 mL of concentrated acetic acid, 85 mL of methanol, and 5 mL of concentrated sulfuric acid. Column chromatography (CC) at atmospheric pressure: glass columns; the stationary phases used in this type of CC are: polyamide CC6, particle size 0.05–0.16 mm (Sigma-Aldrich, Bornem, Belgium); 50 CC at very low pressure: glass columns and nitrogen under pressure (1.2 bar); the stationary phases used in this type of (CC) are: silica gel, class-high purity, pore size 60 Å, 230–400, particle size 40–60 µm (Sigma-Aldrich); MCI gel CHP20P, particle size 75–150 µm (Mitsubishi Chemical Co., Japan); Diaion HP-20. Flash chromatographic purification and isolation was performed on a Reveleris® X2 system (Grace, Columbia, MD, USA) using Reveleris® Navigator™ software. The system consists of a binary pump capable of mixing up to four solvents, an ultraviolet (UV) detector, an evaporative light scattering detector (ELSD), and a fraction collector. Silica gel columns Claricept Flash, particle size 40–60 µm, 60 Å 120 g, and 80 g were used, in which the samples for analysis are applied in dry form, and a Claricept Flash Spherical C18 column with particle size 40–60 µm, 100 Å 80 g, in which the samples for analysis are applied in liquid. Fractionation is based on simultaneous UV and ELSD detection. Mass spectra were obtained by LC-HRESI-MS analysis with a Q Exactive Plus mass spectrometer coupled to a Dionex UltiMate 3000 LC system (Thermo Fischer Scientific, Germering, Germany). Sample recording was performed under both positive and negative ionization. The full MS scan lasts 18.5 min with a run time of 0.5 to 19.0 min, resolution 70 000, AGC target 3e6, max. IT 100 ms, scan range 150 to 1500 m/z. The MS/MS scan was set to resolution 17 500 and AGC target 1e5, maximum IT 50 ms, scan range 200 to 2000 m/z, isolation window 2.0 m/z, and step (N)CE 10, 30, 60 The following parameters were used: dry gas flow (N_2 8.0 L/min, capillary temperature 320 °C, source temperature 320 °C, sheath gas flow – 36 AU, auxiliary flow – 11 AU, source voltage -3.5 kV, and capillary voltage -320 V. Data acquisition and processing were performed using Thermo Xcalibur 2.2 software (Thermo Fischer Scientific Inc., Waltham, MA, USA).Optimum separation was performed on a Zorbax SB-C18 inverted column phase (150 mm x 2.1 mm inner diameter; 3.5 µm particle size) (Agilent Technologies, Waldbronn, Germany) under gradient elution conditions using a binary mobile phase composed of 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The following gradient program was used: 0 min, 5% B; 11 min, 40% B; 17.5 min, 95% B; and 19.5 min, 95% B. The mobile phase flow rate was 0.3 mL/min, and the column temperature was set at 40.0 °C. The injection volume is 2.5 µL. ^{1}H and ^{13}C-NMR spectra were taken on a Bruker Avance II+ 600 with a BVO head at 600.13 and 150 MHz, respectively, in C_5D_5N (99.5%, Deutero GmbH). For correlated spectroscopy (COSY), reflecting the 1H-1H correlations; heteronuclear single quantum coherence (HSQC); reflecting the direct correlation of ^{1}H-^{13}C; and heteronuclear multiple bond correlation (HMBC), reflecting the direct correlation of ^{1}H-^{13}C on long, standard pulses were used programs and phase cycles. NMR data were recorded and processed by Bruker Topspin 4.0.7 software.

Extraction and isolation

The air-dried and ground leaves of the species *C. longipes* (660 g) were extracted with 70% MeOH (8 L) in an ultrasonic bath. After filtration and evaporation with a vacuum evaporator, a total of 42 g dry extract was obtained. This extract was dissolved in water (0.1% FA) and fractionated using normal pressure chromatography with Diaion HP-20 sorbent. Elution was performed gradually, using a gradient from H_2O to MeOH with increasing percentages of MeOH. This process resulted in the collection of six fractions: 0%, 10%, 30%, 50%, 70%, and 90%. In the initial stage of purification, a Reverelis X2 chromatographic system was employed. A pre-packed column with silica gel, having a particle size of 40–60 µm and a pore size of 60Å, weighing 120 g (ClaricepTMSi C-Series, 120 g), was used for the separation. The 90% fraction was prepared by suspending it in silica gel (Kieselgur) before application. The elution process was carried out in a gradient across four steps, with a flow rate of 5 mL/min. Detection was

performed using both ELSD and UV at 254 nm, resulting in the collection of 414 fractions, each 10 mL in volume. Subsequent TLC analysis led to the combination of these fractions into 21 subfractions. Fraction number 17 underwent further purification using normal pressure column chromatography. A column with dimensions of 35 cm by 3 cm and polyamide CC6 sorbent (20.9 g) was utilized. Gradient elution was performed from $CHCl_3$ to MeOH in steps, collecting fractions with a volume of 20 mL each. This process resulted in 41 fractions, which, after TLC analysis, were combined into the subfractions 90_17A, 90_17B, 90_17C, 90_17D, and 90_17E. Fraction 90_17B was chromatographed using the Reveleris X2 system with a pre-packed C18 column, 40 μm (12 g) (FlashPure BUCHI). The fraction was prepared for elution by dissolving it in MeOH (50:50). Elution was performed in three steps at a flow rate of 10 mL/min, collecting 220 fractions of 4 mL each, which were combined into six fractions through TLC analysis. Each of the six fractions underwent further chromatographic purification using a semi-preparative HPLC system. This process utilized an Ascentis® C18 column (250 mm x 15 mm, 5 μm) (Supelco) under isocratic elution for 50 minutes with a 40% acetonitrile (0.1% FA). As a result, three compounds were isolated. Their structures were elucidated by 1D and 2D NMR experiments (COSY, HSQC, and HMBC) as well as LC-HRESI-MS analysis.

Chemistry

19*α*-hydroxyasiatic acid was isolated as a white powder (1.9 mg), and during LC-HRESI-MS analysis, it was observed as a protonated molecule $[M+H]^+$ at *m/z* 503.3387, corresponding to the molecular formula $C_{30}H_{47}O_6$. NMR spectral data for 19*α*-hydroxyasiatic acid: ^{1}H NMR (C_5D_5N) δ, ppm: 0.88 (s, 3H, $-CH_3$), 1.04 (s, 3H, $-CH_3$), 1.13 (d, 3H, *J*=6.55, $-CH_3$), 1.14 (s, 3H, $-CH_3$), 1.29; 2.35 (m, 2H, $-CH_2-$), 1.35, 2.08 (m, 2H, $-CH_2-$), 1.41; 1.83 (t, 2H, $-CH_2-$), 1.43 (s, 3H, $-CH_3$), 1.50 (t, 1H, −CH=), 1.61; 1.40 (m, 2H, $-CH_2-$), 1.68 (s, 3H, $-CH_3$) 1.83, 1.95 (t, dd, 2H, *J*=4.47, 12.25, $-CH_2-$), 2.06, 3.11 (m, td, 2H, *J*=4.84; 13.53, $-CH_2-$), 2.08, 2.17 (m, 2H, $-CH_2-$), 2.11 (m, 1H, −CH=), 2.12, 2.19 (m, 2H, $-CH_2-$), 2.19 (m, 1H, −CH=), 3.06 (brs, 1H, −CH=), 3.76, 3.94 (d, 2H, *J*=10.77; 10.77, $-CH_2OH$), 4.16 (d, 1H, *J*=2.69, −CH=), 4.30 (dt, 1H, *J*=3.27; 10.95, −CH=), 5.61 (t, 1H, Ar, −CH=) (Suppl. material 1: figs S2–C4). ^{13}C NMR (C_5D_5N) δ, ppm: 16.96 (C^{30}, $-CH_3$), 17.19 (C^{25}, $-CH_3$), 17.42 (C^{26}, $-CH_3$), 17.89 (C^{24}, $-CH_3$), 18.53 (C^6, $-CH_2-$), 24.31 (C^{11}, $-CH_2-$), 24.88 (C^{27}, $-CH_3$), 26.37 (C^{16}, $-CH_2-$), 27.08 (C^{21}, $-CH_2-$), 27.29 (C^{29}, $-CH_3$), 29.39 (C^{15}, $-CH_2-$), 33.27 (C^7, $-CH_2-$), 38.78 (C^{22}, $-CH_2-$), 39.3 (C^{10}, =C=), 40.82 (C^8, =C=), 42.10 (C^{14}, =C=), 42.28 (C^4, =C=), 42.69 (C^{20}, −CH=), 42.90 (C^1, $-CH_2-$), 43.75 (C^5, −CH=), 47.86 (C^9, −CH=), 48.80 (C^{17}, =C=), 54.82 (C^{18}, −CH=), 66.42 (C^2, −CH=), 71.32 (C^{23}, $-CH_2OH$), 73.09 (C^{19}, =C=), 79.09 (C^3, −CH=), 128.20 ($C^{12}{}_{arom}$), 140.00 ($C^{13}{}_{arom}$), 180.00 (−COOH) (Suppl. material 1: figs S6, S7).

1*α*,3*β*,23-trihydroxyolean-12-en-28-oic acid was isolated as white powder (5.3 mg) and during LC-HRESI-MS analysis was observed as a deprotonated molecule $[M-H]^-$ at *m/z* 487.3437, corresponding to the molecular formula $C_{30}H_{47}O_5$. NMR spectral data for 1*α*,3*β*,23-trihydroxyolean-12-en-28-oic acid: ^{1}H NMR (C_5D_5N) δ, ppm: 0.93 (s, 3H, $-CH_3$), 1.00 (s, 3H, $-CH_3$), 1.06 (s, 3H, $-CH_3$), 1.08 (s, 3H, $-CH_3$), 1.08, 1.95 (m, 2H, $-CH_2-$), 1.09 (s, 3H, $-CH_3$), 1.15, 1.43 (m, 2H, $-CH_2-$), 1.21 (s, 3H, $-CH_3$), 1.27, 1.76 (m, 2H, $-CH_2-$), 1.32, 1.67 (m, 2H, $-CH_2-$), 1.35, 2.18 (m, 2H, $-CH_2-$), 1.42, 2.32 (d, dd, 2H, *J*=11.03; 4.10, 12.44, $-CH_2-$), 1.46, 1.75 (m, 2H, $-CH_2-$), 1.82, 2.05 (m, 2H, $-CH_2-$), 1.85 (m, 1H, −CH=), 1.93 (m, 1H, −CH=), 2.05 (m, 2H, $-CH_2-$), 3.30 (dd, 1H, *J*=4.21, 13.96, −CH=), 3.75, 4.23 (m, 2H, $-CH_2-$), 4.23 (dd, 1H, *J*=4.12, 10.43, −CH=), 4.27 (dd, 1H, *J*=4.07, 9.6, −CH=), 5.48 (t, 1H, Ar, −CH=) (Suppl. material 1: figs S9–S11). ^{13}C NMR (C_5D_5N) δ, ppm: 14.53 (C^{24}, $-CH_3$), 17.58 (C^{25}, $-CH_3$), 17.66 (C^{26}, $-CH_3$), 18.69 (C^6, $-CH_2-$), 23.77 (C^{11}, $-CH_2-$), 23.93 (C^{30}, $-CH_3$), 24.38 (C^{16}, $-CH_2-$), 26.31 (C^{27}, $-CH_3$), 28.49 (C^{15}, $-CH_2-$), 31.00 (C^{20}, =C=), 32.98 (C^7, $-CH_2-$), 33.29 (C^{29}, $-CH_3$), 34.40 (C^{21}, $-CH_2-$), 38.50 (C^{10}, C), 40.00 (C^8, =C=), 42.20 (C^{18}, −CH=), 42.33 (C^{14}, =C=), 44.30 (C^4, =C=), 46.00 (C^{17}, =C=), 46.67 (C^{19}, $-CH_2-$), 47.82 (C^2, $-CH_2-$), 48.05 (C^5, −CH=), 48.35 (C^9, =C=), 66.61 (C^{23}, $-CH_2-$), 69.08 (C^{22}, $-CH_2-$), 69.08 (C^1, −CH=), 78.60 (C^3, −CH=), 122.77 ($C^{12}{}_{arom}$), 145.53 ($C^{13}{}_{arom}$), 180.20 (−COOH) (Suppl. material 1: figs S13, S14).

Rosamultic acid was isolated as a white powder (1.4 mg), and during LC-HRESI-MS analysis, it was observed as a deprotonated molecule $[M-H]^-$ at *m/z* 485.3273, corresponding to the molecular formula $C_{30}H_{45}O_5$. NMR spectral data for rosamultic acid: ^{1}H NMR (C_5D_5N) δ, ppm: 1.12 (d, 3H, *J*=6.56, $-CH_3$), 1.16 (s, 3H, $-CH_3$), 1.25 (s, 3H, $-CH_3$), 1.35, 2.08 (m, 2H, $-CH_2-$), 1.43 (s, 3H, $-CH_3$), 1.49 (s, 3H, $-CH_3$), 1.50 (m, 1H, −CH=), 1.73 (s, 3H, $-CH_3$), 1.82 (s, 1H, −CH=), 2.07, 2.14 (m, 2H, $-CH_2-$), 2.38, 2.48 (m, 2H, $-CH_2-$), 2.59 (m, 1H, −CH=), 3.05 (m, 1H, −CH=), 3.86, 4.06 (m, 2H, *J*=10.45; 10.45, $-CH_2OH$), 4.50, 4.64 (d, 2H, *J*=15.45; 15.45, $-CH_2OH$), 5.62 (m, 1H, −CH=), 6.12 (s, 1H, −CH=) (Suppl. material 1: fig. S16). ^{13}C NMR (C_5D_5N) δ, ppm: 16.93 (C30, $-CH_3$), 18.59 (C6, $-CH_2-$), 18.80 (C26, $-CH_3$), 19.63 (C25, $-CH_3$), 24.30 (C11, $-CH_2-$), 25.26 (C24, $-CH_3$), 25.56 (C27, $-CH_3$), 25.74 (C16, $-CH_2-$), 27.22 (C29, $-CH_3$), 27.30 (C21, $-CH_2-$), 28.56 (C15, $-CH_2-$), 33.43 (C7, $-CH_2-$), 39.00 (C22, $-CH_2-$), 42.32 (C14, =C=), 42.32 (C20, −CH=), 43.82 (C8, =C=), 44.12 (C9, −CH=), 48.54 (C17, =C=), 49.70 (C4, =C=), 51.20 (C10, =C=), 55.08 (C18, −CH=), 61.03 (C1, $-CH_2OH$), 63.94 (C5, −CH=), 66.57 (C23, $-CH_2OH$), 73.09 (C19, =C=), 128.30 (C12, −CH=), 131.72 (C3, −CH=), 140.76 (C13, =C=), 158.59 (C2, =C=), 180.10 (−COOH) (Suppl. material 1: figs S18, S19).

Genotoxicity test

Saccharomyces cerevisiae is a useful eukaryotic model to study the cellular response to different compounds and stressors (Delaunay et al. 2000; Zimmermann et al. 2018; Lingua et al. 2019). This microorganism is particularly beneficial in studies involving biological activity, as it helps overcome ethical obstacles (Ruta et al. 2020). The stress response in this organism is well researched, similar

to higher organisms, including human responses to oxidative stress. (Jamieson 1998; Wright et al. 2014; Marinovska et al. 2022). Yap1 is a crucial factor that regulates oxidative stress, such as exposure to H_2O_2 (Ruta et al. 2020). The YAP1 transcription factor in *S. cerevisiae* is a functional homolog of mammalian AP-1 (Maeta et al. 2004; Maeta et al. 2004). The regulation of oxidative stress is mainly related to the factor's translocation from the cytosol to the nucleus, followed by the induction of the transcription of antioxidant genes (Delaunay et al. 2000). Two strains of *S. cerevisiae* were used in this study: 551 rho+ with the genotype MATα, ura3, his3Δ200, sec53, rho+ (Pesheva et al., 2005) and 551yap1Δ with the genotype MATα, ura3, his3Δ200, sec53, yap1Δ (Stoycheva et al. 2010). Both strains possess the mutant allele ts1 (SEC53), which leads to insufficient glycosylation of mannoproteins in the cell wall and increases its permeability (Pesheva et al. 2005). The 551yap1Δ strain has a deletion of the yap1 gene, which disables the synthesis of the Yap1 protein and consequently impairs the peroxide neutralization system (Stephen et al. 1995). Cell suspensions were cultivated under standard conditions at 30 °C and 200 rpm until the end of the exponential growth phase. Treatment was performed for 30 min under optimal conditions with compounds 1, 2, and 3 (Cl-01, Cl-03, and ClA-06) at concentrations ranging from 0.001 to 0.1 μg/mL. Post-treatment, cell suspensions were diluted and plated on YEPD medium (1% yeast extract, 2% peptone, 2% dextrose) to assess cell survival.

Statistical analysis

Data analysis was conducted using GraphPad Prism software, version 9.5.1 (San Diego, USA). Differences among strains, tested concentrations, and positive controls were evaluated using one-way ANOVA with Bonferroni's multiple comparisons test and two-way ANOVA with Tukey's multiple comparisons test. Significance levels were indicated as follows: *P < 0.05; **P < 0.01; ***P < 0.001. Each test was performed in at least three independent experiments.

Result and discussion

Identification and characterization of isolated pentacyclic triterpenoids

Compound **1** was isolated as a white crystalline powder (1.9 mg). The data from HRESI-MS analysis shows a protonated molecule ion $[M+H]^+$ at *m/z* 503.3387, corresponding to the molecular formula $C_{30}H_{47}O_6$. The NMR assignments of the compound match those of the *β*-glycosyl ester of 19*α*-hydroxyursolic acid, isolated from *Symplocos spicata* (Higuchi et al. 1982). The ^{13}C NMR spectrum showed 30 signals, including six methyl groups d 17.89 (C-24), 17.19 (C-25), 17.42 (C-26), 24.88 (C-27), 27.29 (C-29), and 16.96 (C-30), two secondary OH groups d 66.42 (C-2), 79.09 (C-3), one tertiary d 73.09 (C-19), and one double bond d 128.2 (C-12), 140.0 (C-13). The ^1H NMR spectrum showed signals for one secondary methyl group at 1.13 (d, *J* 6.55 Hz, 3H, CH_3), five tertiary methyl groups d 0.88 (s, 3H, CH_3), 1.04 (s, 3H, CH_3), 1.14 (s, 3H, dCH_3), 1.68 (s, 3H, CH_3), and 1.43 (s, 3H, CH_3), an ethylene proton at d 5.61 (t, 1H, H-12), and a proton at d 3.06 (brs, 1H, H-18) belonging to H-18, indicating the presence of a Δ12-ursene skeleton. The presence of four OH groups was confirmed by signals in the ^1H NMR spectrum for two secondary OH groups d 4.30 (dt, *J* 3.27; 10.95 Hz, 1H, H-2), d 4.16 (d, *J* 2.69 Hz, H-3), one tertiary d 73.09 (C-19), and one primary OH group d 3.76 and 3.94 (d, *J* 10.77 Hz, 2H, H-23), belonging to H-23, similar to asiatic acid, due to the carbon resonance of C-24 at d 17.89. The doublet at d 4.16 (d, *J* 2.69 Hz, H-3) belongs to H-3, linked in the COSY spectrum with a signal at d 4.30 (dt, *J* 3.27, 10.95 Hz, H-2), belonging to H-2. Therefore, after a full analysis of the COSY, HSQC, and HMBC spectra, compound **1** was identified as 2*α*,3*β*,19*α*,23-tetrahydroxy-urs-12-en-28-oic acid (19*α*-hydroxyasiatic acid) (Seto et al. 1984). Based on literature data examination, this compound was isolated for the first time from the genus and species *Cecropia*. Compound **2** was isolated as a white crystalline substance (5.3 mg). High-resolution HRESI-MS analysis showed a deprotonated molecule $[M-H]^-$ at *m/z* 487.3437, corresponding to the molecular formula $C_{30}H_{47}O_5$. NMR spectral data for compound **2** matched those of the pentacyclic triterpene 1*α*,3*β*,23-trihydroxyolean-12-en-28-oic acid, previously isolated from *Lithospermum caroliniense* (Fullas et al. 1996). The ^{13}C NMR spectrum displayed 30 signals, attributed to six methyl, ten methylene, six methine, and seven quaternary carbons. The olean-12-ene triterpene skeleton was confirmed by the chemical shifts of d 122.77 (C-12) and d 145.53 (C-13), as well as the proton signal of H-18 at d 3.30 (dd, *J* 4.21 and 13.96 Hz, H-18) and the triplet signal of H-12 at d 5.48 (t, 1H, Ar). In the ^1H NMR spectrum, two doublets (1H each) at d 3.75 and 4.23 (m, *J* 10.0 Hz, H-23) were assigned to the hydroxymethyl group at C-23, while the signal at d 4.23 (dd, *J* 4.12 and 10.43 Hz, H-3) suggested an equatorial orientation of the hydroxyl group at C-3. In the COSY NMR spectrum, the oxymethine protons of C-1 and C-3 were correlated with the methylene protons of C-2. The substitution pattern in ring A was confirmed by observing HMBC correlations of H-2a d 2.32 (dd, *J* 4.10; 12.44 Hz, H-2a) with d 69.08 (C-1) and d 78.6 (C-3), H-3 d 4.23 (dd, *J* 4.12, 10.43 Hz, H-3) with C-1, and d 3.75 (m, 1H, H-23a) with d 14.53 (C-24) and C-3. Further analysis of the HMBC and HSQC spectra allowed the assignment of the remaining ^{13}C NMR signals. Based on these observations, the structure of compound **2** was identified as 1*α*,3*β*,23-trihydroxyolean-12-en-28-oic acid. According to literature data, this compound was first isolated from the genus and species *Cecropia*. Compound **3** was isolated as white powder (1.4 mg). High-resolution HRESI-MS analysis showed a deprotonated molecule $[M-H]^-$ at *m/z* 485.3273, corresponding to the molecular formula $C_{30}H_{45}O_5$. The close similarity in NMR assignments between compound **3** and compound **1** strongly supports the presence of the Δ12-ursene skeleton in compound **3**. Compound 3 is presented as an A-ring contracted terpene, whereas C-1 d 61.03 is presented as a hydroxymethyl

group. This statement is confirmed also by the HMBC correlations between d 4.50 (d, *J* 15.45 Hz, 1H, H-1a) and 4.64 (d, J 15.45 Hz, 1H, H-1b) with d 131.72 (C-3). Comparing the observed NMR assignments with literature data, compound **3** was described as rosamultic acid (Yeo et al. 1998).

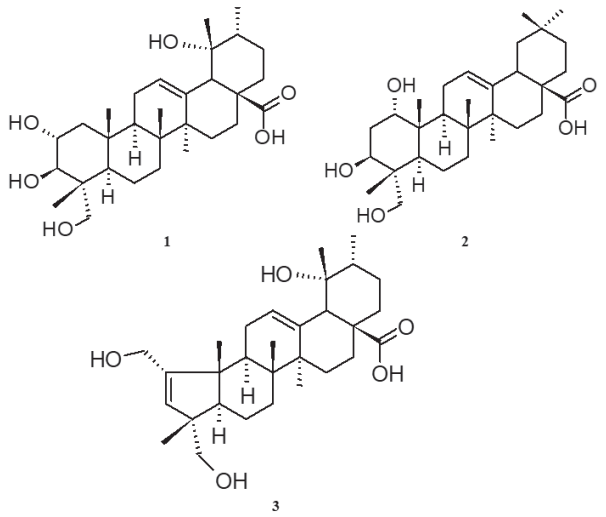

Figure 1. Structure of compounds **1**–**3**.

Genotoxicity test results

The biological activity of compounds 1, 2, and 3 was evaluated using the *S. cerevisiae* model system to assess their potential genotoxic effects. The analysis using *S. cerevisiae* indicated that both compounds 2 and 3 demonstrated genotoxic effects across all tested concentrations. In contrast, compound 1 did not affect cell survival at concentrations of 0.001 and 0.01 µg/mL. However, genotoxicity was observed at higher concentrations of 0.05 and 0.1 µg/mL (Fig. 2).

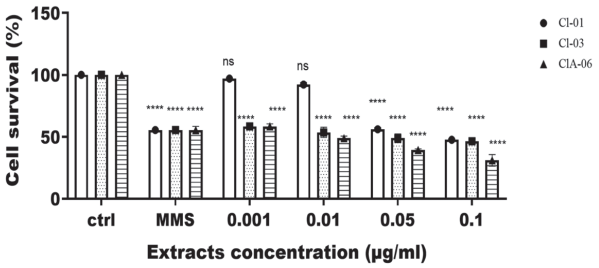

Figure 2. Genotoxic effects of compounds 1, 2, and 3 (Cl-01, Cl-03, and ClA-06).

To determine if the antioxidant defense system was involved in the genotoxic activity of compound 1, we compared results between the 551 rho+ strain and the 551Δyap1 strain. Although significant differences were observed between the effects on the two strains, the variance was around 10% (Fig. 3).

This suggests that the genotoxic effects of compound 1 may not be primarily due to oxidative stress but could be due to other mechanisms.

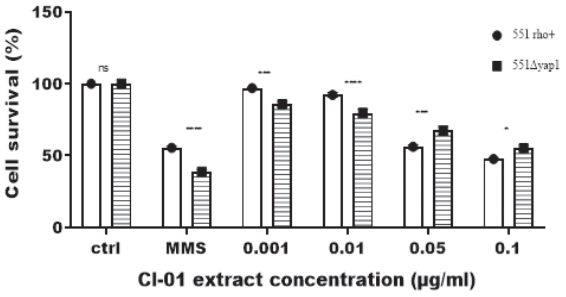

Figure 3. Genotoxic activity of compound 1 for the 551 rho+ strain and the 551Δyap1 strain.

Conclusion

During the research, a phytochemical study of the leaves of *C. longipes* was carried out to investigate the presence of pentacyclic triterpenoids. Using a series of chromatographic methods on a 70% MeOH extract of the leaves, three compounds were isolated. The results of the ^{1}H NMR analysis indicated the presence of a pentacyclic triterpenoid structure in the isolated substances. The structures of the three compounds were determined by 1D (^{1}H NMR, ^{13}C NMR) and 2D NMR (COSY, HSQC, HMBC) as 19*α* hydroxyasiatic acid, 1*α*,3*β*,23-trihydroxyolean-12-en-28-oic acid, and rosamultic acid. These compounds were isolated for the first time from the genus *Cecropia* and the species *C. longipes*. Additionally, a genotoxicity test was conducted on the isolated compounds using the *S. cerevisiae* model system. The results revealed that compounds 2 n and 3 exhibited genotoxic activity at all tested concentrations. For 19*α* hydroxyasiatic acid, no effect on cell survival was observed at concentrations of 0.001 and 0.01 µg/mL, but genotoxic potential was noted at higher concentrations of 0.05 and 0.1 µg/mL. Comparing the results between the 551 rho+ strain and the 551Δyap1 strain suggested that the genotoxicity of 19*α* hydroxyasiatic acid is not strongly related to oxidative stress but likely involves other mechanisms. This finding provides a deeper understanding of the biological activity and safety profile of these newly isolated compounds.

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Supplementary material 1

Supplementary data

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