SUPPLEMENTARY MATERIAL

Phytochemical screening by LC-MS and LC-PDA of ethanolic extracts from the fruits of *Kigelia africana* (Lam.) Benth.

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Abstract: *Kigelia africana* is a tree native to Africa, with a local employment in numerous fields, ranging from traditional medicine to cosmetics and religious rituals. Parts of the plant generally used are stem bark, fruits, roots, and leaves. The fruits, which have a singular "sausage" shape, are widely exploited by local folk, in particular for applications/products involving genito-urinary apparatus of both human genders. The scope of this work was to make a consistent chemical investigation on this plant species, in order to clarify and increase the information at present available in literature. To this aim, ethanolic extracts of *K. africana* fruits were analyzed by high performance liquid chromatography with photodiode array (HPLC-PDA) and electrospray-mass spectrometry (HPLC-ESI-MS) detection, revealing the presence of polyphenols and iridoids. The two detection systems used along with standard co-injection and comparison with previous reports, led to the identification and quantification of six phenolic compounds and three iridoids.

Keywords: *Kigelia africana*; sausage tree; HPLC-ESI-MS; polyphenols; iridoids.

Experimental

Chemicals

Caffeic acid, ferulic acid, p-coumaric acid, minecoside, and verbascoside were purchased from Sigma (Sigma Chemical Co., St. Louis, MO). All solvents used for extraction and analysis by TLC were purchased from VWR International PBI (Italy). Water, methanol, formic acid, and hexane for LC analyses were from Sigma-Aldrich (Milan, Italy).

Plant material

Kigelia africana (Lam) Benth., syn. *K. pinnata* (Bignoniaceae) fruits were collected in the belt of Bamako (Mali), in June 2015. Identification of plants was carried out in the Traditional Medicine Department (TMD), Faculty of Medicine, University of Bamako. A voucher specimen (nr. 03128) was deposited in the Department of Chemical, Biological, Pharmaceutical and Environmental Sciences of the University of Messina (Italy).

Extraction

Three dried fruits obtained from three different trees were powdered and mixed; an aliquot (100 g) of this mixture was subjected to Soxhlet extraction with 625 mL of 60% ethanol for 36 hours. The extract was filtered and concentrated to dryness under vacuum in a rotary evaporator (Buchi R-205), at a temperature of 40°C and a pressure of 337 mbar. The extraction yield was 0.125 w/w.

For LC-PDA and LC-MS analyses, the dried ethanolic extract of Kigelia africana was further subjected to a clean-up procedure: 0.1 g of extract were added with 5 mL of methanol and washed with 10 mL of hexane to remove the apolar fraction. The solution obtained after evaporation at 30°C, and dissolution in 1 mL distilled water, was passed through a C18 SPE cartridge (Sigma Aldrich-Supelco, PA, USA). The polyphenolic fraction was weighed (5 mg) and eluted with 4 mL of methanol, for injection into the HPLC system.

LC analysis

A Shimadzu Nexera X2 system (Shimadzu, Milan, Italy), including a CBM-20A communication bus module, two LC-30 AD dual-plunger parallel-flow pumps, a DGU-20A5R on-line degasser, a CTO-20AC column oven and a SIL-30AC autosampler, was employed for LC separations. An SPD-M30A PDA detector equipped with a highly sensitive flow cell and a triple quadrupole LCMS-8040, with ESI interface (Shimadzu,

Milan, Italy), were employed, respectively, for the quantification and characterization of analytes.

For analytes separation, an Ascentis Express C18 (250 x 4.6 mm I.D. x 2.7 µm. d.p.) (Supelco, Bellefonte, PA, USA) was used. Mobile phases consisted of water/formic acid (99.9:0.1,v/v) (solvent A) and methanol/formic acid (99.9:0.1,v/v) (solvent B). Analyses took place in gradient mode: 0.01 min 2% B; 7 min, 7% B; 60 min, 60% B; 75 min, 100% B. The flow-rate was 1.0 mL/min and the temperature of column oven was set at 30°C. Injection volume was 5.0 µL. The wavelength range for PDA acquisition was 200-600 nm and chromatograms were extracted at 280 nm for polyphenols, and at 240 and 330 nm, for iridoids. Time constant and sample frequency were respectively 0.60 s and 1.5625 Hz. MS acquisition was performed using the ESI interface operating in the negative ionization mode, according to the following conditions: mass spectral range, 100-800 m/z; event time, 0.15 sec; scan speed, 5000 amu/s; nebulizing gas (N_2) flow: 2.0 L/min; drying gas (N_2) flow, 15.0 L/min; heat block temperature, 250°C; desolvation line (DL) temp., 250°C; interface voltage, 3.5 kV; detector voltage, 1.80 kV. The effluent from the column was split before entering the MS instrument (approximately 0.3 mL/min to the MS). Data acquisition was performed by *Lab solution LCMS* (ver. 5.53 SP2) software (Shimadzu, Japan).

Figure S1 – HPLC-PDA chromatogram of ethanolic extract of *Kigelia africana*. Peak#1: caffeic acid glucoside; peak#2: p-coumaroyl glucose; peak#3: caffeic acid; peak#4: p-coumaric acid; peak#5: ferulic acid; peak#6: verbascoside; peak#7: verminoside; peak#8: unkown 1; peak#9: unknown 2; peak#10: specioside; peak#11: minecoside.

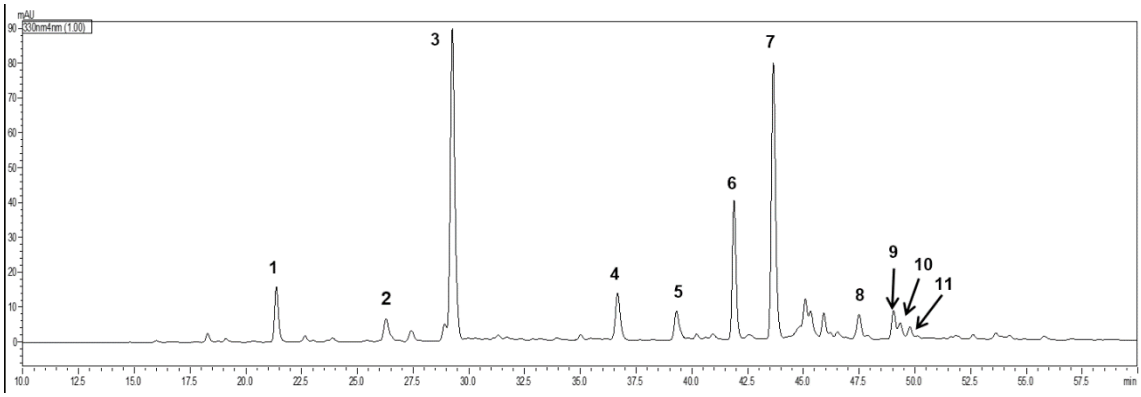

Figure S2 - LC-ESI$^-$ mass spectra and LC-PDA spectra of compounds *unknown 1* (A) and *unknown 2* (B).