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Title:

Anthelmintic activity of extracts and active compounds of *Diospyros anisandra* on *Ancylostoma* spp., *Haemonchus* spp. and cyathostomins

Actividad antihelmíntica de extractos y compuestos activos de *Diospyros anisandra* en *Ancylostoma* spp., *Haemonchus* spp. y cyathostomins

Knowledge area: **Agricultural sciences and biotechnology**

Abstract:

¹H- NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer at 400 MHz. The CG-MS was carried out on an Agilent Technologies (model 6890N) instrument using the following chromatographic conditions: split injection of 1 µL of a 1% concentration sample; an Ultra1 column (25 m × 0.2 mm i.d), flow rate of 1.0 mL min⁻¹ (helium as the carrier gas) and an oven temperature program of T1 = 180 °C (3 min) and T2 = 280 °C (15 min), a gradient of 10 °C/min and an injector and detector temperature (FID) of 280 °C.

Keywords: Active compounds, bioguided fractionation, gastrointestinal nematodes, plant extracts, plumbagin.

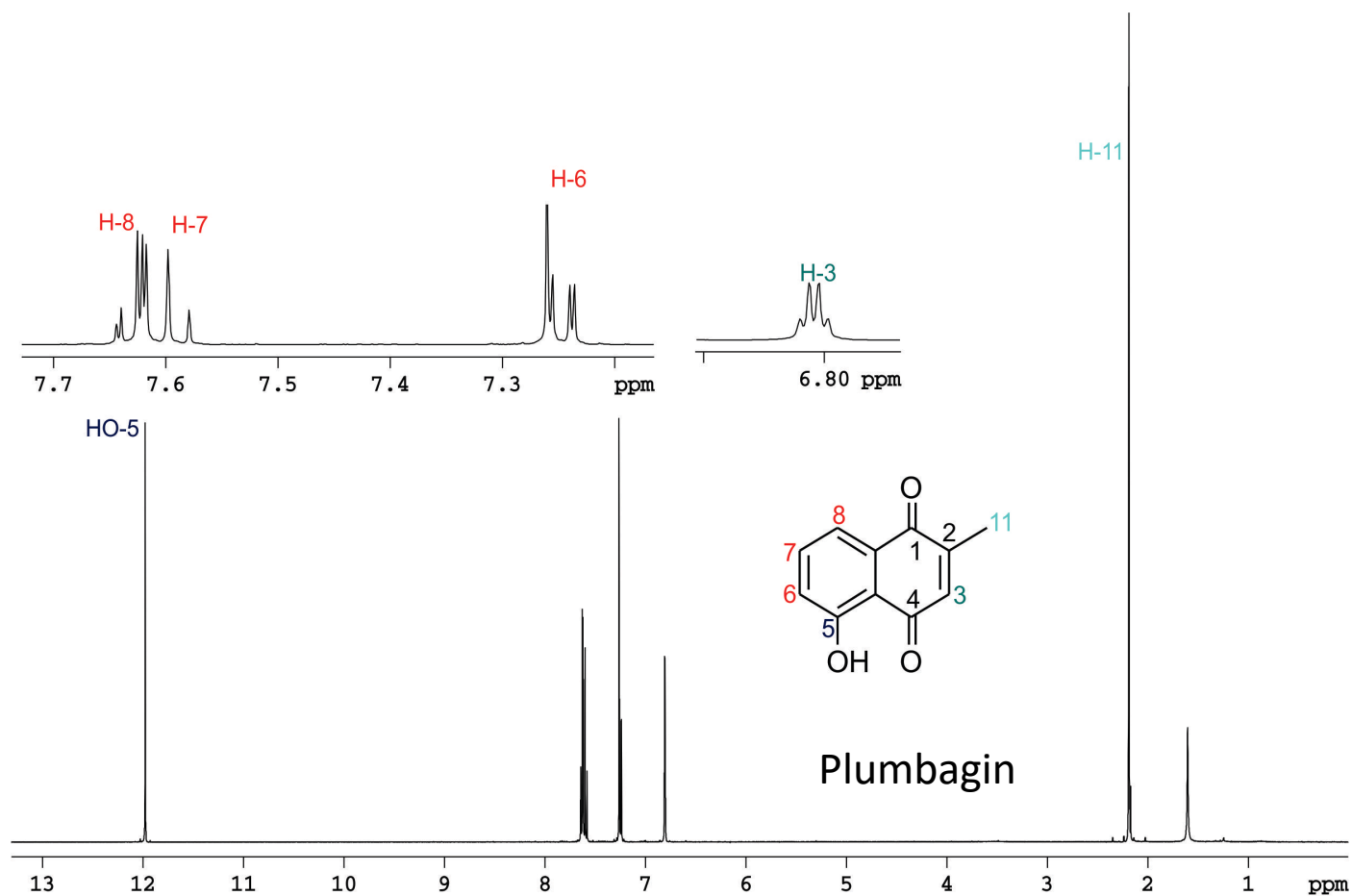


Fig 1S. ¹H NMR of Plumbagin at 400 MHz in CDCl₃

Plumbagin (5-hidroxi-2-metil-1,4-naftalendiona): Orange-yellow needles; melting point. 74-75 °C. ¹H NMR (400 MHz, CDCl₃): δ 2.18 (3H, d, $J=1.5$ Hz, H-11), 6.80 (1H, q, $J=1.5$ Hz, H-3), 7.24 (1H, dd, $J=7.8, 1.7$ Hz, H-6), 7.60 (1H, t, $J=7.8$ Hz, H-7), 7.63 (1H, dd, $J=7.8, 1.7$ Hz, H-8), 11.97 (1H, s, OH-5) (Mallavadhani *et al.*, 1998).

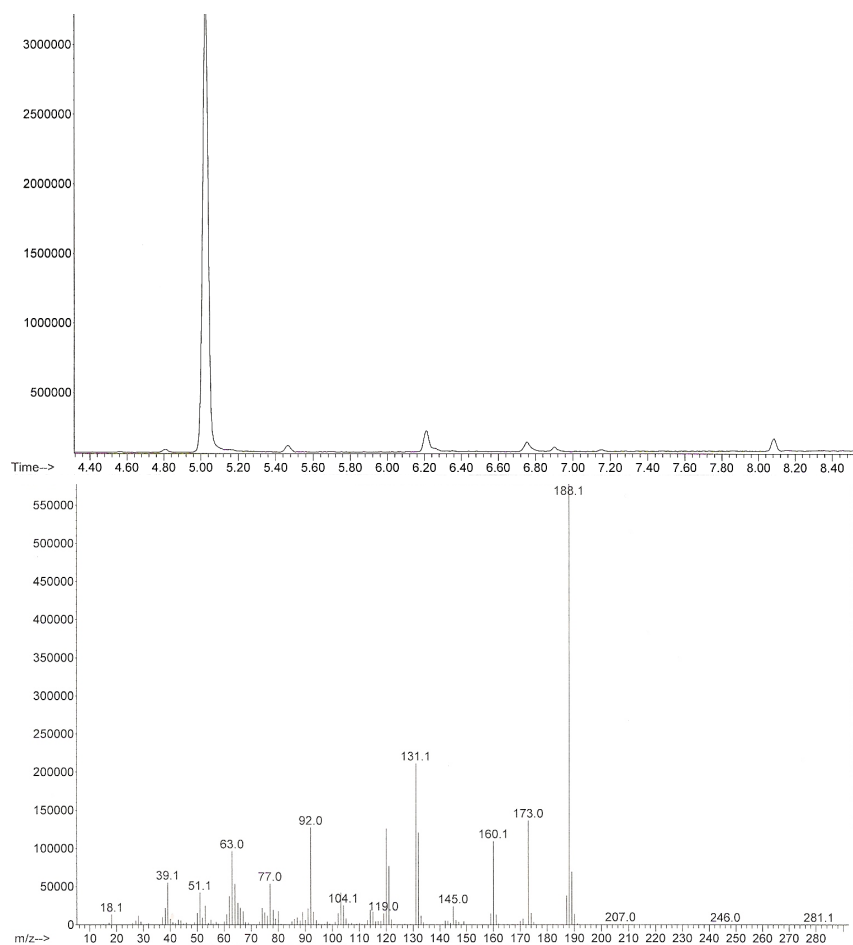


Figure 2S: Chromatographic profile of Plumbagin and mass fragmentation pattern.

GC-MS of Plumbagin at RT= 5.02 min; molecular ion at m/z 188.1 (Figure 2S) and main fragments at m/z 173 $[M - CH_3]^+$, 160 $[M - CO]^+$, 131 $[M - CO - HCO]^+$, 120 $[M - C_3H_4 - CO]^+$, 92 $[C_6H_4O]^+$ (Figure 3S) (Stensen & Jensen, 1995).

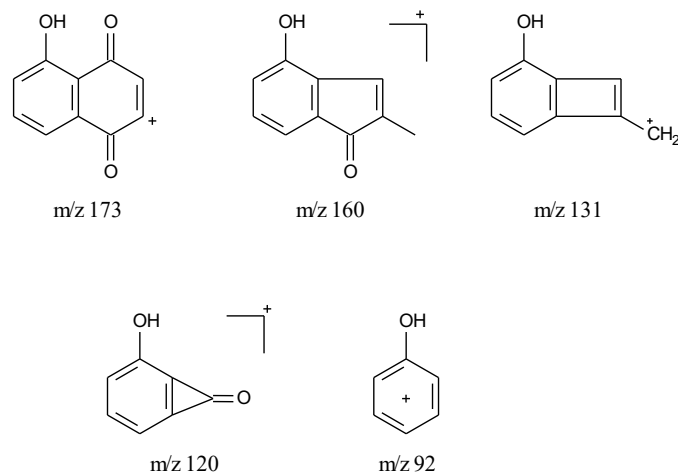


Figure 3S. Main mass fragments of Plumbagin

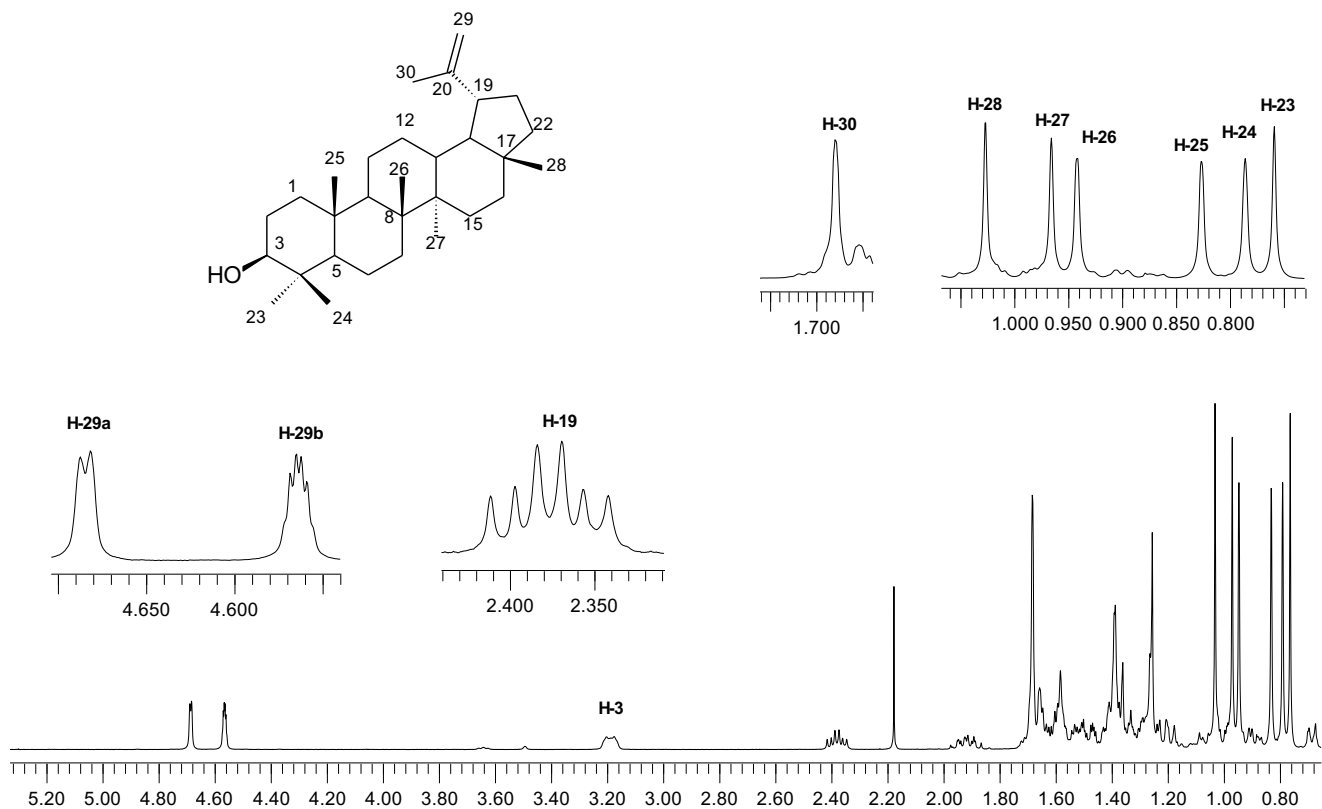


Figure 4S: ^1H NMR of Lupeol at 400 MHz in CDCl_3

Lupeol: White powder; melting point: 214-215 °C; ^1H NMR (400 MHz, CDCl_3): δ 0.75 (3H, s, H-23), 0.78 (3H, s, H-24), 0.82 (3H, (3Hs, H-25), 0.94 (3H, s, H-26), 0.98 (3H, s, H-27), 1.02 (3H, s, H-28), 1.67 (3H, s, H-30), 2.37 (1H, dt, $J= 11.0, 5.8$, H-19), 3.18 (1H, m, H-3), 4.56 (1H, dd, $J= 2.4, 1.3$ Hz, H-29b), 4.68 (1H, d, $J= 2.3$ Hz, H-29a) (Fotie et al., 2006; Seger et al., 1997).

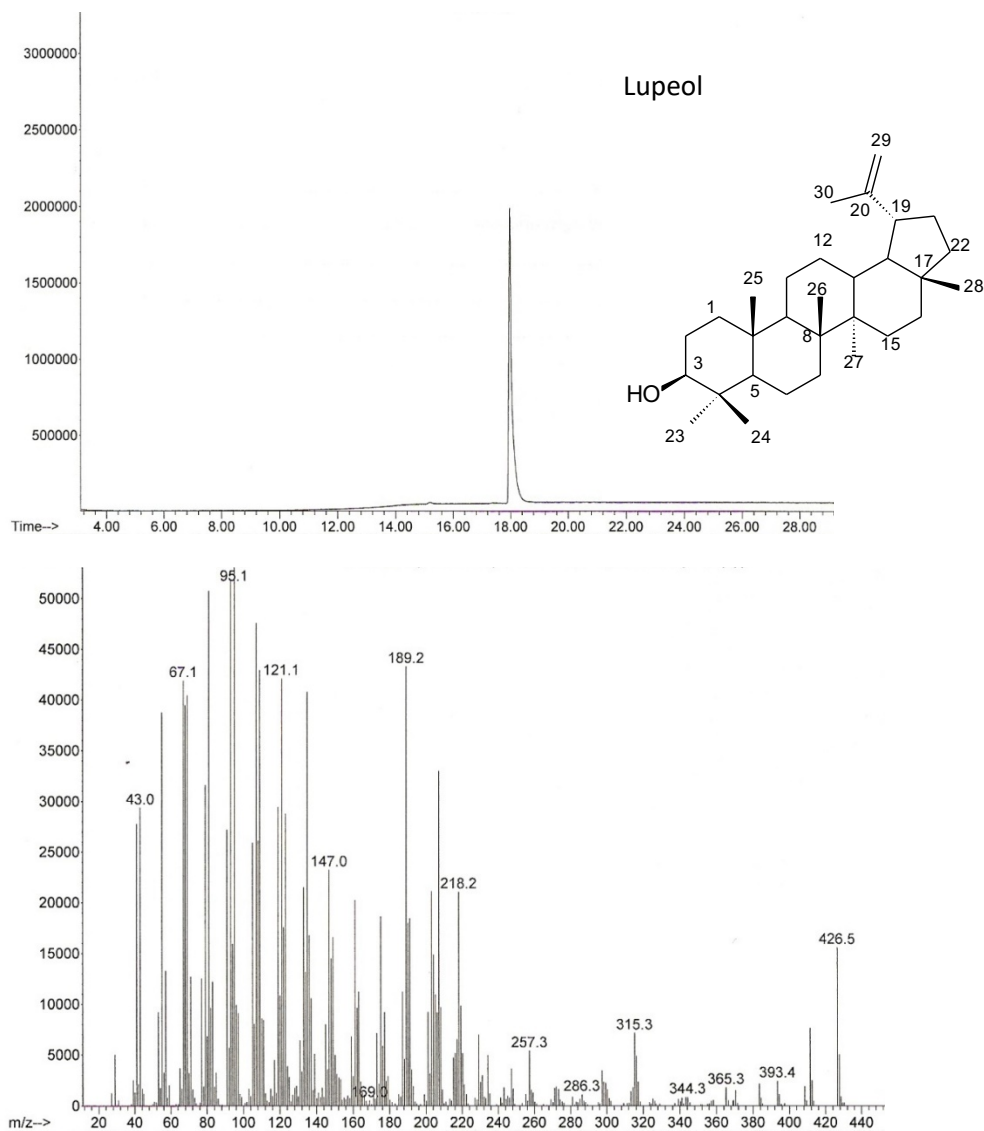


Figure 5S: Chromatographic profile of Lupeol and mass fragmentation pattern.

GC-MS of Lupeol at RT= 18.00 min; molecular ion at m/z 426.5 (Figure 5S) and main fragments at m/z 189 and 218 (Figure 6S) (Khan *et al.*, 1980).

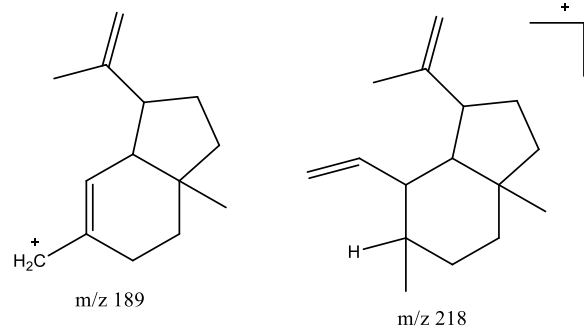


Figure 6S. Main mass fragments of Lupeol

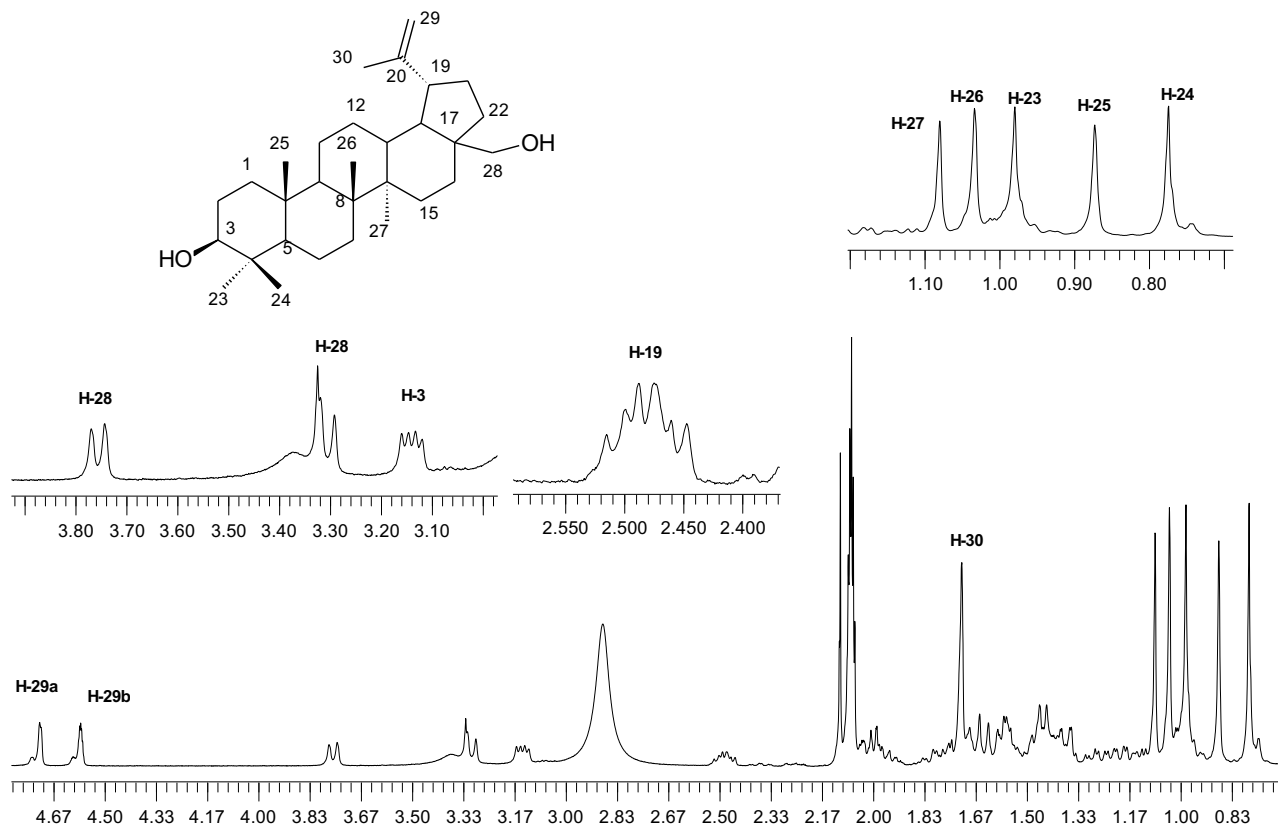


Figure 7S: ¹H NMR of Betulin at 400 MHz in CDCl₃

Betulin: White powder; melting point: 258-259 °C; ¹H NMR (400 MHz, CDCl₃): δ 0.77 (3H, s, H-24), 0.87 (3H, s, H-25), 0.97 (3H, s, H-23), 1.03 (3H, s, H-26), 1.07 (3H, s, H-27), 1.70 (3H, s, H-30), 2.47 (1H, dt, *J* = 10.7, 5.2, H-19), 3.13 (1H, dd, *J* = 10.8, 5.2 Hz, H-3), 3.30 (1H, d, *J* = 4.62 Hz, H-28a); 3.75 (1H, d, *J* = 10.4 Hz, H-30b), 4.57 (1H, bs, H-29b), 4.71 (1H, bs, H-29a) (Jin *et al.*, 2007).

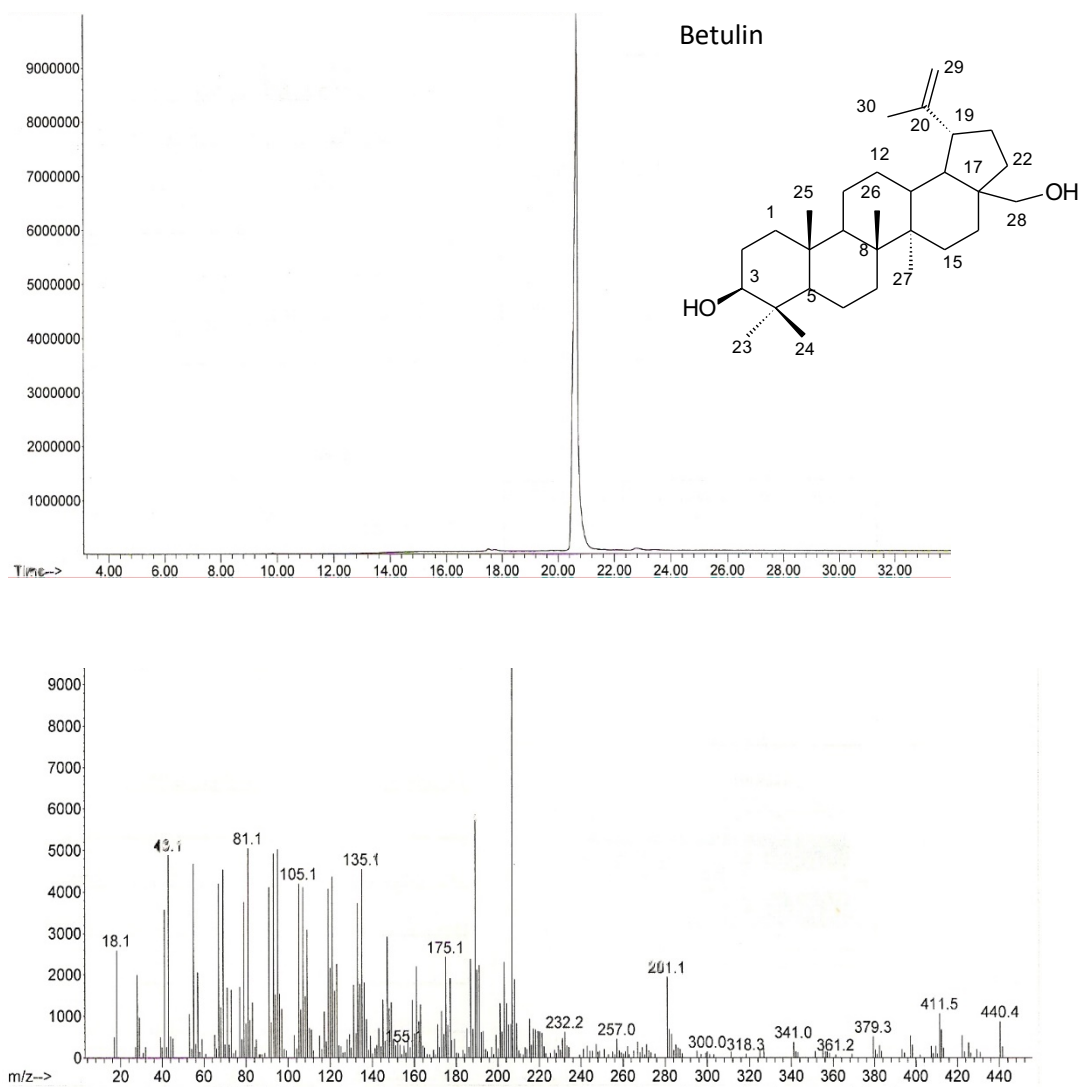


Figure 8S: Chromatographic profile of Betulin and mass fragmentation pattern.

The compound exhibited a retention time of 20.6 min with a molecular ion of m/z 440.4 (Figure 8S); characteristic fragments of a lupane-like skeleton are observed in the MS spectrum at m/z 189, 218, 220 and 232 (Figure 9S) (Ogunkoya, 1980).

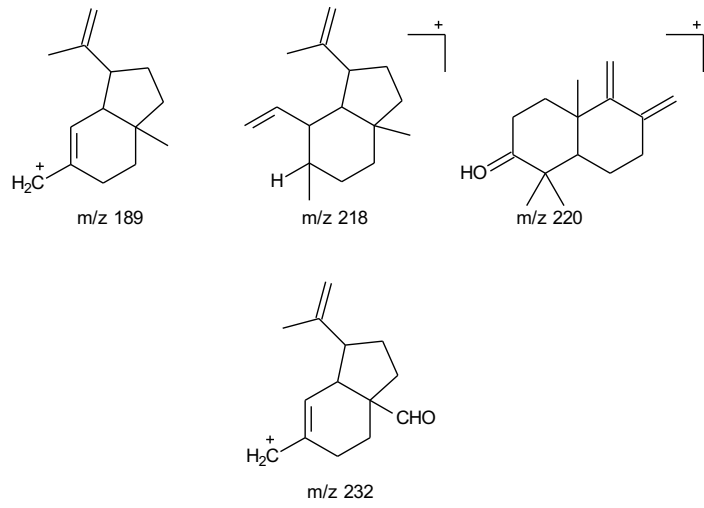


Figure 9S. Main mass fragments of Betulin

References

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