SUPPLEMENTARY MATERIAL

Establishment and Phytochemical Analysis of a Callus Culture from *Ageratina pichinchensis* (Asteraceae) and Its Anti-Inflammatory Activity

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Abstract

A protocol was established to produce bioactive compounds in a callus culture of *Ageratina pichinchensis* by using 1 mg L^{-1} NAA with 0.1 mg L^{-1} KIN. The phytochemical study of the EtOAc extract obtained from the callus biomass, allowed the isolation and characterization of eleven secondary metabolites, of which dihydrobenzofuran (**5**) and 3-epilupeol (**7**), showed important anti-inflammatory activity. Compound **5** inhibits *in vitro* the secretion of NO (IC_{50} = 36.96 ± 1.06 μM), IL-6 (IC_{50} = 73.71 ± 3.21 μM), and TNF (IC_{50} = 73.20 ± 5.99 μM) in RAW 264.7 macrophages, as well as the activation of NF-κB (40 % at 150 μM) in RAW-blue macrophages, while compound **7** has been described that inhibit the *in vivo* TPA-induced ear edema, and the in vitro production of NO, and the PLA2 enzyme activity. In addition, quantitative GC-MS analysis showed that the anti-inflammatory metabolites **5** and **7** were not detected in the wild plant. Overall, our results indicated that *A. pichinchensis* can be used as an alternative biotechnological resource for obtaining anti-inflammatory compounds. This is the first report of the anti-inflammatory activity of compound **5** and its production in a callus culture of *A. pichinchensis*.

Keywords: *Ageratina pichinchensis*; dihydrobenzofuran; 3-epilupeol; callus culture; anti-inflammatory.

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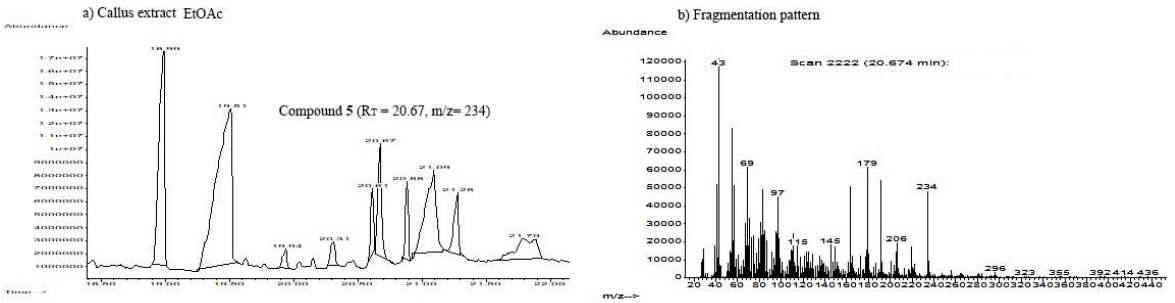


Figure S1. GC-MS analyses of **5** in the EtOAc extract of callus, identification of compound was obtained by analysis of the peak at R_T = 20.67 min.

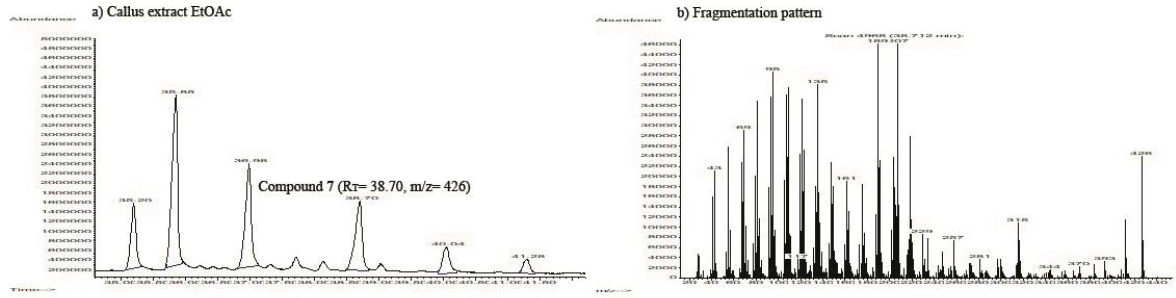


Figure S2. GC-MS analyses of **7** in the EtOAc extract of callus, identification of compound was obtained by analysis of the peak at R_T = 38.7 min.

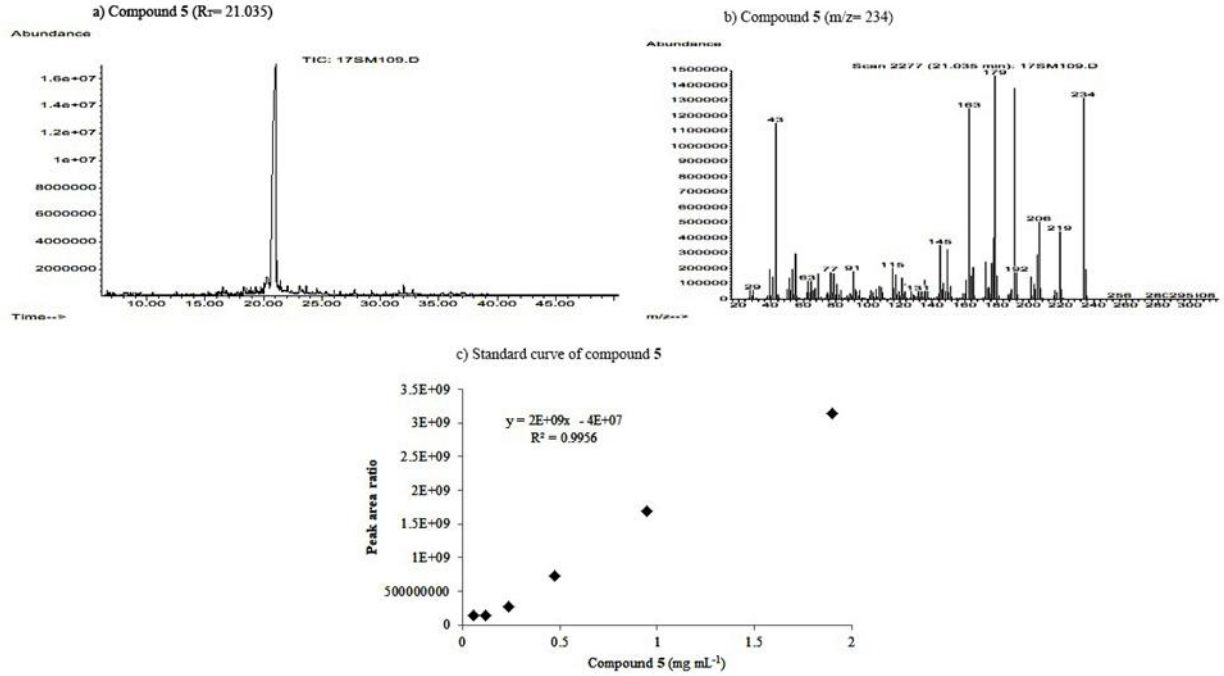


Figure S3. GC-MS analysis of pure compound **5.**

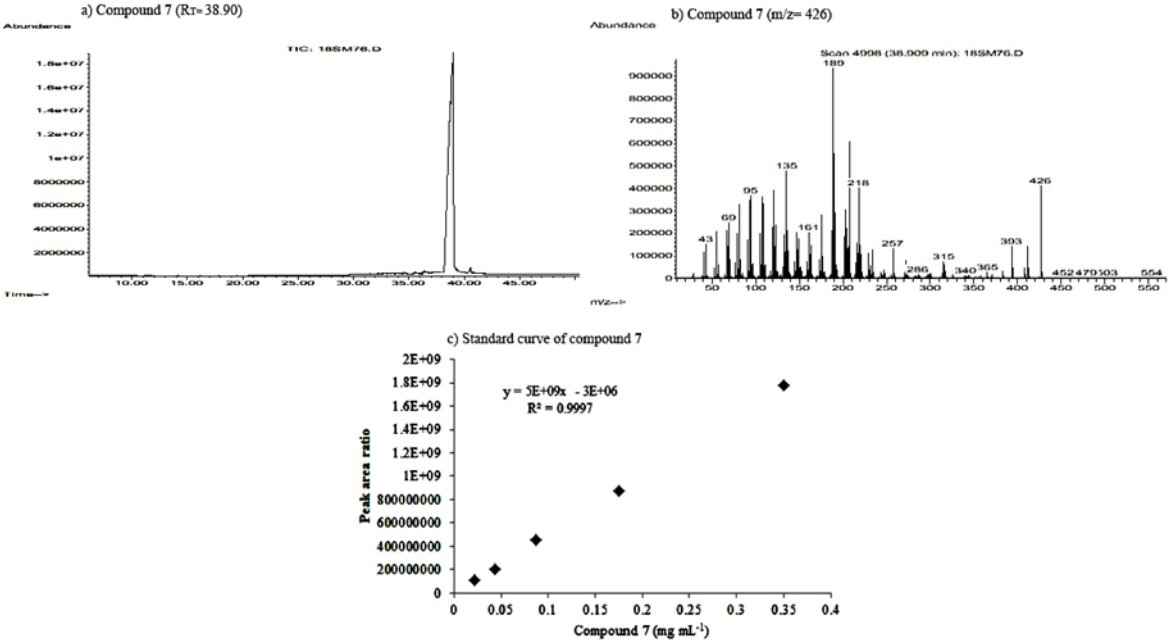


Figure S4. GC-MS analysis of pure compound **7**.

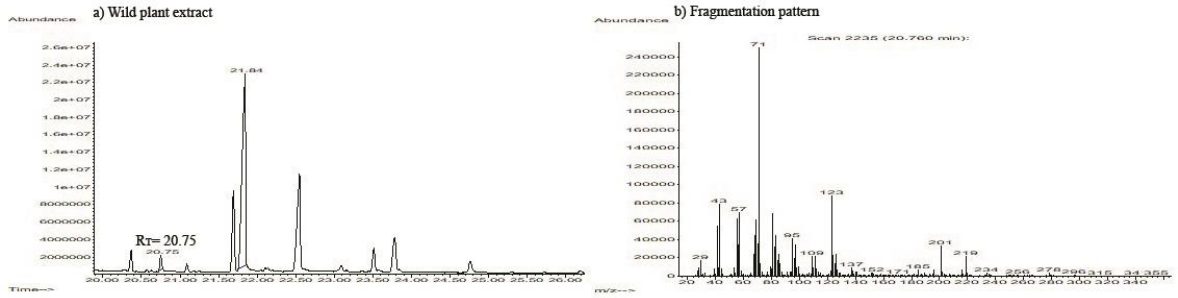


Figure S5. GC-MS analysis of compound **5** in wild plant.

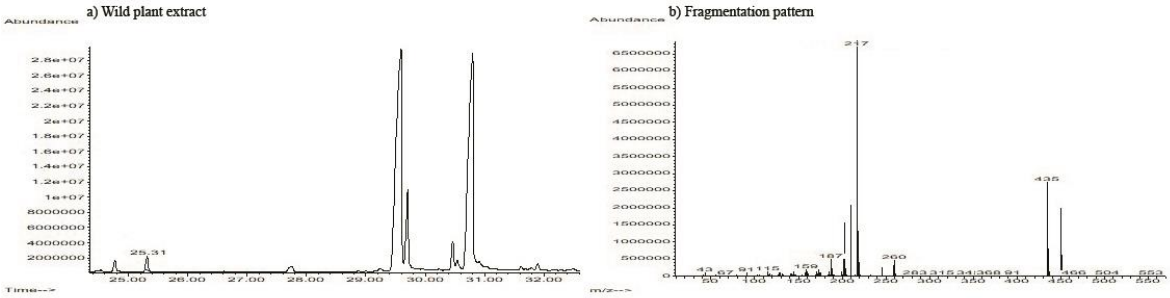


Figure S6. GC-MS analysis of compound **7** in wild plant.

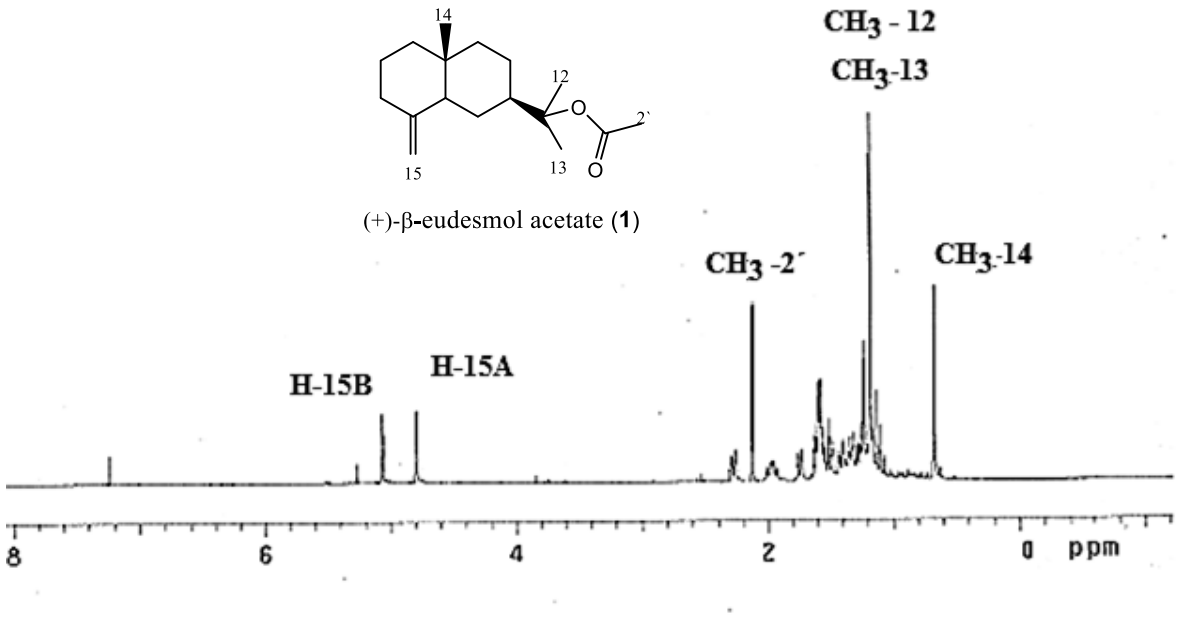


Figure S7. ^{1}H NMR spectrum (400 MHz; $CDCl_3$) of compound **1.**

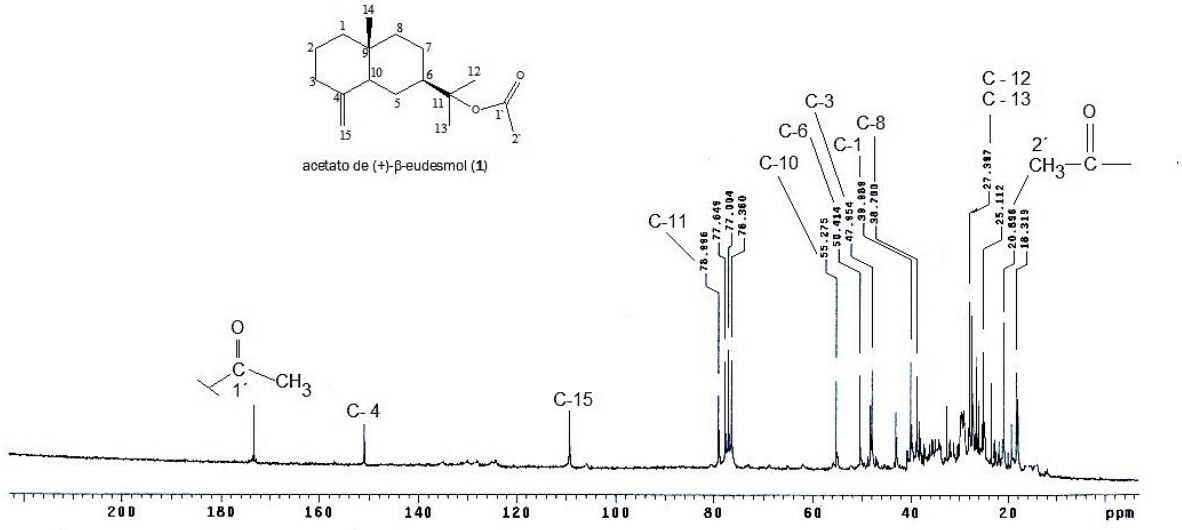


Figure S8. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **1.**

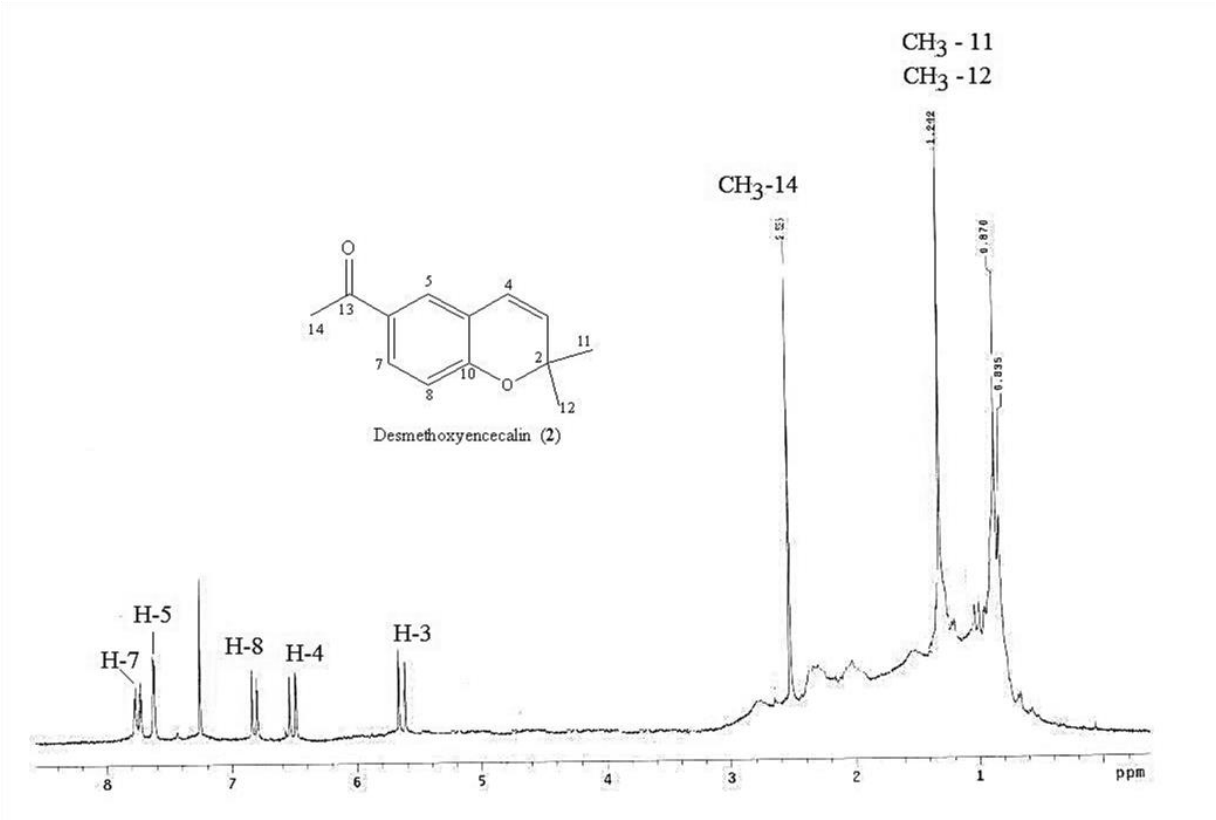


Figure S9. ^{1}H NMR spectrum (400 MHz; $CDCl_3$) of compound **2.**

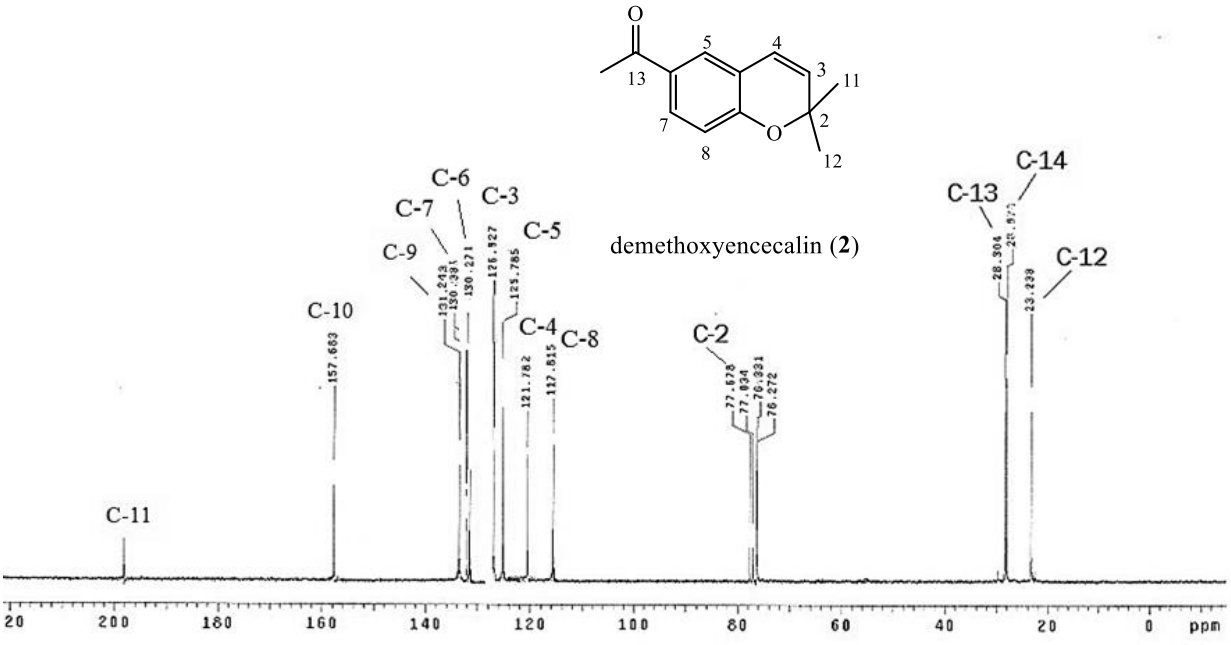
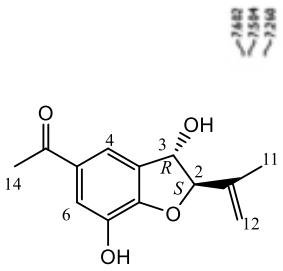


Figure S10. ^{13}C NMR spectrum (100 MHz, CDCl3) of compound **2.**



5-acetyl-7,3α-dihydroxy-2β-(1-isopropen)-2,3-dihydrobenzofuran (**5**)

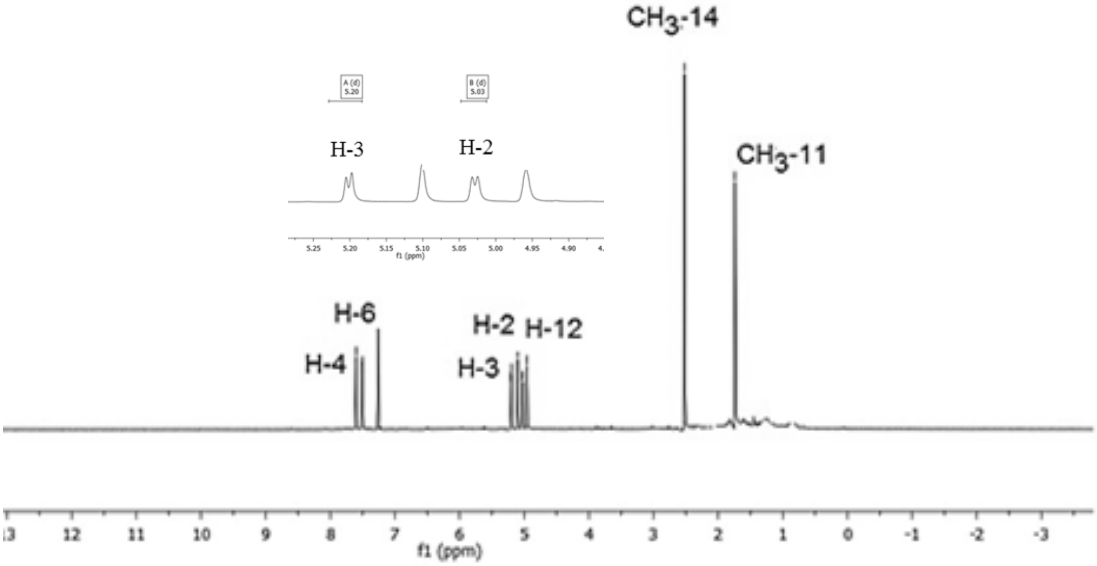


Figure S11. ^{1}H NMR spectrum (500 MHz; $CDCl_3$) of compound **5.**

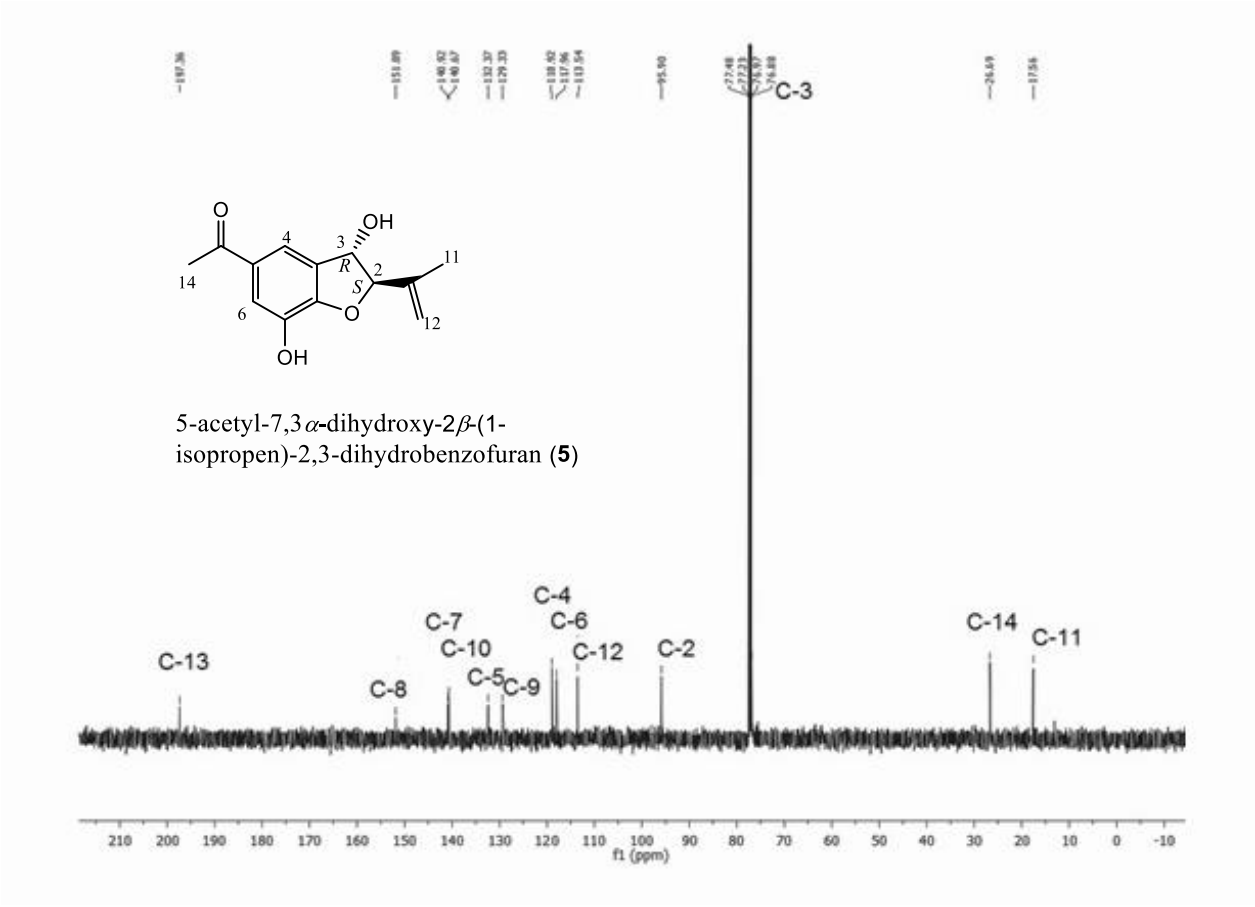


Figure S12. ^{13}C NMR spectrum (125 MHz, CDCl3) of compound **5.**

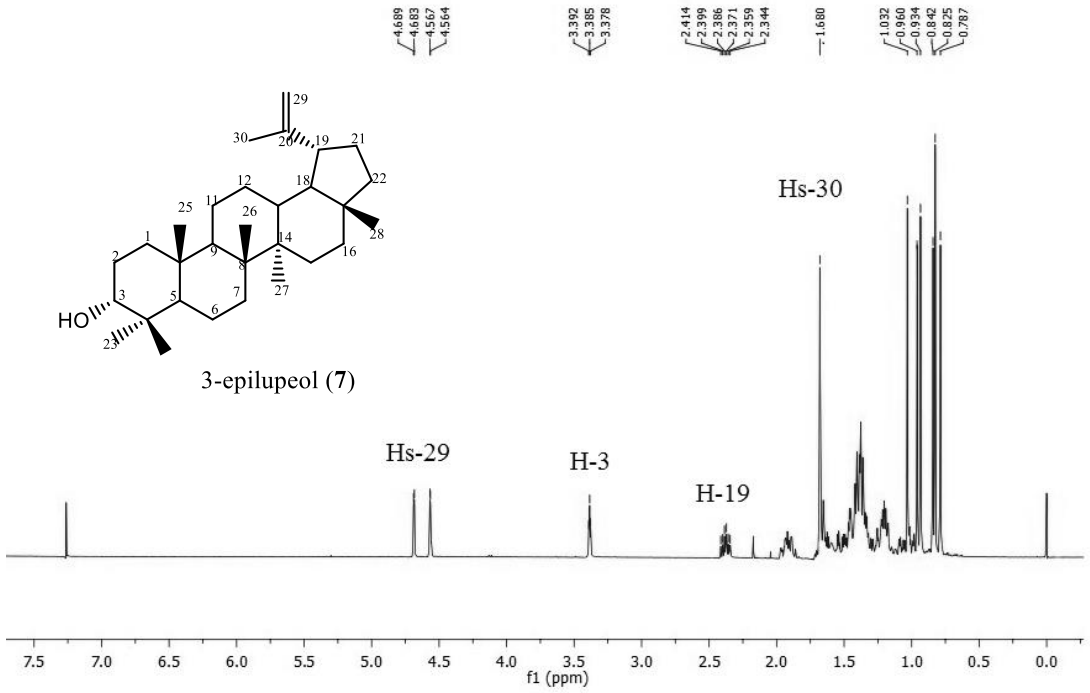


Figure S13. ^{1}H NMR spectrum (400 MHz; $CDCl_3$) of compound **7.**

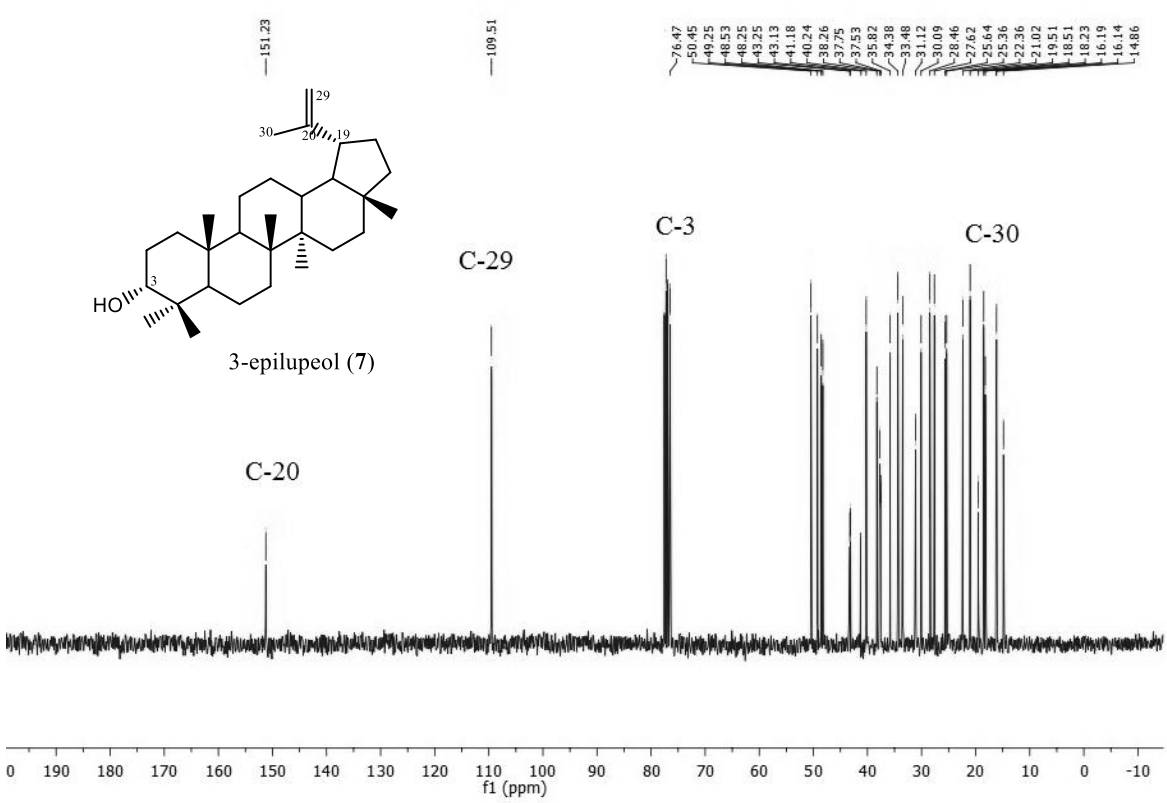


Figure S14. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **7.**