

Chromatographic Process to Obtain Galloylquinic Acid Compounds from *Copaifera langsdorffii* Desf

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Purpose

Isolate galloylquinic acid compounds from *Copaifera langsdorffii* Desf., which presents antilithiasic activity, using chromatographic techniques.

Methods

Hydroalcoholic extract was prepared by macerating 1kg of *C. langsdorffii* leaves with alcohol 70%. Partitioning was done with solvents of increasing polarities. The resulted fractions were analyzed by thin layer chromatography (TLC) and high performance liquid chromatography with ultraviolet diode-array detector (HPLC-UV-DAD) in a binary gradient system, consisted of A: acidified water (0.1% of formic acid) and B: methanol, in a flow rate of 1mL/min. In order to isolate galloylquinic acid compounds from aqueous fraction it was used sephadex gel filtration chromatography, with 300g of Sephadex™ LH20. Five grams of aqueous fraction were eluted in the column, collecting 420 subfractions of 20mL each. The subfractions that presented simpler chromatographic profiles were purified in a preparative HPLC. The isolated substances structures were confirmed using a Nuclear Magnetic Resonance (NMR) technique.

Results

From 1kg of *C. langsdorffii* leaves it was obtained 340g of hydroalcoholic extract, 34% of yield. The partitioned hydroalcoholic extract (50 g) yielded fractions of hexane (1.4%), dichloromethane (2.1%), ethyl acetate (34.4%), n-butanolic (31.7%) and aqueous (28.8%), which demonstrates the presence of high polarity compounds. It was also possible to verify the chromatographic profile of the extract and fractions by HPLC-UV-DAD in 280nm, a specific wavelength for galloylquinic acids, and it was noticed that they were present mainly in the n-butanolic and aqueous fractions. For that, 5.0g of aqueous fraction was eluted in a Sephadex™ column, resulting in 420 fractions that, afterwards, were gathered by TCL similarities in 35 subfractions. Using preparative HPLC it was possible to purify two subfractions, furnishing two substances that according to NMR analyses were: 5''-O-methyl-3,4-di-O-galloylquinic acid (31.4mg) and 5',5'-di-O-methyl-3,4-di-O-galloylquinic acid (29.2mg) (Figure 1).

Conclusion

C. langsdorffii is majorly constituted by polar compounds, mainly galloylquinic acids. In the present work, it was possible to obtain and identify two of them from the aqueous fraction, enabling the future biologic activity studies.

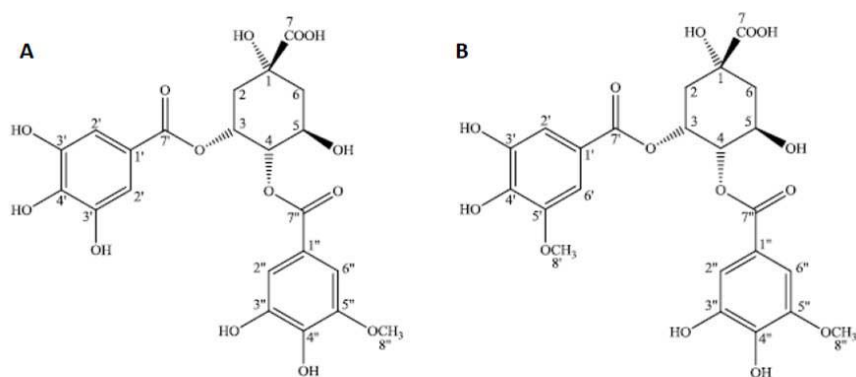


Figure 1: Galloylquinic acid compounds from *Copaifera langsdorffii* Desf. **A:** 5''-O-methyl-3,4-di-O-galloylquinic acid **B:** 5',5'-di-O-methyl-3,4-di-O-galloylquinic acid.