PRODUCTION OF MIMONOSIDES BY TRANSFORMED ROOTS OF *MIMOSA TENUIFLORA*

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Introducción. *Mimosa tenuiflora* (Willdenow) Poiret is a spontaneous Fabaceae distributed from Mexico to Brazil, the bark of which is used in the Mexican traditional medicine for treating burns and skin inflammation. Its overexploitation in Mexico after the San Juanica 1982 natural gas explosion and the 1985 earthquake has lead to the need of developing alternative *in vitro* production systems (1). Many chemical constutuents are accumulated in the bark of the tree: polyphenols, toxic tryptamine derivatives (2) and saponins. The healing effect of this drug is related to the potential cell proliferating and immunostimulant effect of its saponoside content and mainly to three triterpenoid saponins (3) (mimonosides A, B, and C). The saponins are glycosides of triterpenes steroids, or steroid alkaloids that are widely found in plants and some marine organisms. They exhibit a wide range of biological activities (4).

Metodología. Extraction and purification of mimonosides from the bark of the tree: *Mimos*a bark powder was extracted by 70% methanol under reflux. The extract was then concentrated and washed with acetone. The precipitate obtained was redissolved in water, alkalinized, and then extracted with butanol. The butanolic phase which contains saponins was evaporated to dryness. The residue was resuspended in methanol and ether was then added. The resulting precipitate was submitted to chromatography on silica support and elution was performed with a solvent made up of a chloroform gradient, methanol and water. The fractions obtained were finally purified by semi–preparative HPLC.
Mimonosides analytical procedures: mimonosides were identified and analysed by TLC and LC-MS. A gradient procedure was used for the separation of the several saponins present in the extracts and the purified fractions.
Production of *M tenuiflora* hairy roots: hairy root lines of *Mimosa tenuiflora* were obtained by inoculation of micropropagated plantlet leaves with two different *Agrobacterium rhizogenes* strains (15834 and LBA 9402).

Resultados y discusión.
The fractionation of the bark extract allowed the LC-MS identification of mimonosides A, B and C as well as several isomers (4 for mimonoside A, 3 for minonoside B and 2 for mimonoside C) The various analyses carried out allowed to conclude that the protocol of extraction was efficeient and reproducible.
Several root lines were developped from *Agrobacteria* transfections. Root line R3 obtained with bacterial strain 15834 gave rise to fast growing roots (fig 1). High biomass production was achieved by growing them in the temporary immersion system (RITA system®/CIRAD), as well as in a 2 L bioreactor. The secondary metabolite content of the root material and the culture medium was analysed by TLC and LC-MS, and compared to the saponoside fraction of the tree's bark. It was seen that mimonoside A was accumulated in the cultures.

Fig. 1. Hairy roots of Mimosa tenuiflora grown in a flask

Conclusiones.
It was possible to develop an efficient and reproducible protocol to separate and identify mimonosides from the bark of *Mimosa tenuiflora* tree.
Agrobacterium transfection allowed the obtention of *M tenuiflora* fast growing hairy roots. First analyses of root extracts and culture media revealed that at least mimonoside A was accumulated .
This biotechnological procedure will next be optimised and scaled-up as a potential alternative to bark extraction for an industrial production of mimonosides.

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