Triterpenic acids from *Ziziphus jujuba* fruits: quali-quantitative determination and evaluation of their capability to interfere in macrophages activation

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Several studies confirm the notion that a diet rich in fruits and vegetables contributes to the reduction of inflammation and is preventive against related diseases,[1] such as the development of age-related degenerative diseases.[2] The health benefits of plant-based foods are attributed to bioactive phytochemicals, including triterpenoids and other groups of natural compounds, acting in an additive and synergistic manner. All forms of triterpenoids, free or as saponins, are widely distributed in edible and medicinal plants, and consequently, they have to be an integral part of the human diet.[3] Triterpenoids have been shown to possess several biological activities and to exhibit various and interesting pharmacological effects such as anti-inflammatory, antimicrobial, hepatoprotective, antioxidant activities[4] and also antiviral properties (including anti-HIV), combined with relatively low toxicity.[3] *Ziziphus jujuba* Mill (Rhamnaceae) fruits are recognized as an outstanding source of biologically active compounds including triterpenic acids. On the basis of the anti-inflammatory properties attributed to *Z. jujuba* fruits, our investigation on their triterpenic fraction was carried out, yielding nine triterpenic acids (**1**-**9**). Anti-proliferative and anti-inflammatory activity of these compounds was evaluated. To examine the capability of compounds **1**-**9** isolated from *Z. jujuba* to influence cell viability, the isolated compounds **1**-**9** were tested on three different cultured cell lines J774A.1, MCF7 and A459 using the MTT anti-proliferative assay. Cells were treated with graded concentrations of compounds **1**-**9** (10-100 μM) for 72 hours. The obtained results indicated that only compounds **1**, **2**, **5** and **9** did not affect cell viability while the other compounds exerted weak anti-proliferative activities with IC_{50} values ranging from 98 μM to 155 μM. To assess if compounds **1**, **2**, **5** and **9,** unable to affect cell viability, could interfere in LPS-induced J774.A1 macrophages activation, the release of nitrite, a stable end-product of nitric oxide (NO) production in cellular medium of the LPS-activated murine macrophages J774.A1 incubated with compounds **1**, **2**, **5** and **9** (10-100 μM) in combination with LPS (1μg/ml), was measured. Compound **2** (10-100 μM), added to J774 macrophages, 1h before and simultaneously to LPS stimulation, determined a significant ($P<0.01$ vs LPS) and concentration-dependent inhibition of nitrite production in cell medium with an inhibition by 15.44%, 25.23 % and 32.45%, respectively at 10, 25 and 100 μM. Compound **5** (10-100 μM), added to J774.A1 macrophages, 1h before and simultaneously to LPS stimulation, also inhibited significantly ($P<0.01$ vs LPS) and in a concentration-dependent manner nitrite production with an inhibition by 21.34%, 27.27 %, and 37.60%, respectively at 10, 25 and 100 μM. In the same experimental conditions compounds **4** and **7** reduced nitrite production but not significantly neither in a concentration-related way.

In order to perform the quantitative evaluation of the triterpenic derivatives **1**-**9** in *Z. jujuba* fruits, an analytical approach based on liquid chromatography coupled to mass spectrometry with ESI source and triple quadrupole analyzer (LC-ESI(QqQ)MS), using the very sensitive and selective mass tandem experiment called Multiple Reaction Monitoring (MRM), was carried out.

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