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Secondary metabolites production in *in vitro* plants of *Ruta graveolens* (Rutaceae)

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Abstract: Specialized organs, calli and cell suspensions of *Ruta graveolens* cultured *in vitro* produce compounds of pharmacological interest such as alkaloids, coumarins, flavonoids and essential oils [1]. However, there are few studies regarding the production and/or storage sites of these compounds in *R. graveolens in vitro* culture, since they differ from the *ex vitro* plants. We characterized the phytochemical profile of *R. graveolens* leaves from *in vitro* plants. For this, seeds were inoculated into flasks containing MS medium and sealed with rigid polypropylene caps (RPC) without membrane [exchange rate of CO_2 ($ERCO_2$): 14 µL L^{-1} s^{-1}] (treatment 0M) and RPC with a hole (10 mm) covered with a 0.45 µm pore size membrane [$ERCO_2$: 21 µL L^{-1} s^{-1}] (treatment 1M), under 16 h photoperiod and 80 µmol m^{-2} s^{-1} irradiance. After 45days, the ethanol extracts of *in vitro* and *ex vitro* leaves were obtained and the phytochemical characterization by thin layer chromatography (TLC) was performed [2, 3]. For histochemical test, leaf transversal sections of *in vitro* and *ex vitro* plants (0M and 1M) were subjected to Wagner reagent for detection of alkaloids [4]; NADI for essential oils and resins [5]; ferric chloride for phenols [6] and aluminum chloride for flavonoids [7]. Leaves of *in vitro* (0M and 1M) and *ex vitro* plants showed alkaloids, coumarins, antracenic derivates, saponins, flavonoids, triterpenes and steroids. *In vitro* plants showed the evaluated secondary metabolites in the same locations found for *ex vitro* counterparts. Alkaloids and flavonoids were detected only in trichomes and secretory cavities, being more evident in *in vitro* leaves. Non-structural phenolic compounds were detected in palisade and spongy parenchyma. The essential oils were observed in the vascular bundles, in some epidermal cells, in palisade and spongy parenchyma, in the trichomes and secretory cavities. Overall, it can be highlighted that vitroplants of *R. graveolens* have the same biosynthetic capacity of adult plants grown in a greenhouse, varying only in the amount of the secondary metabolites produced.

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