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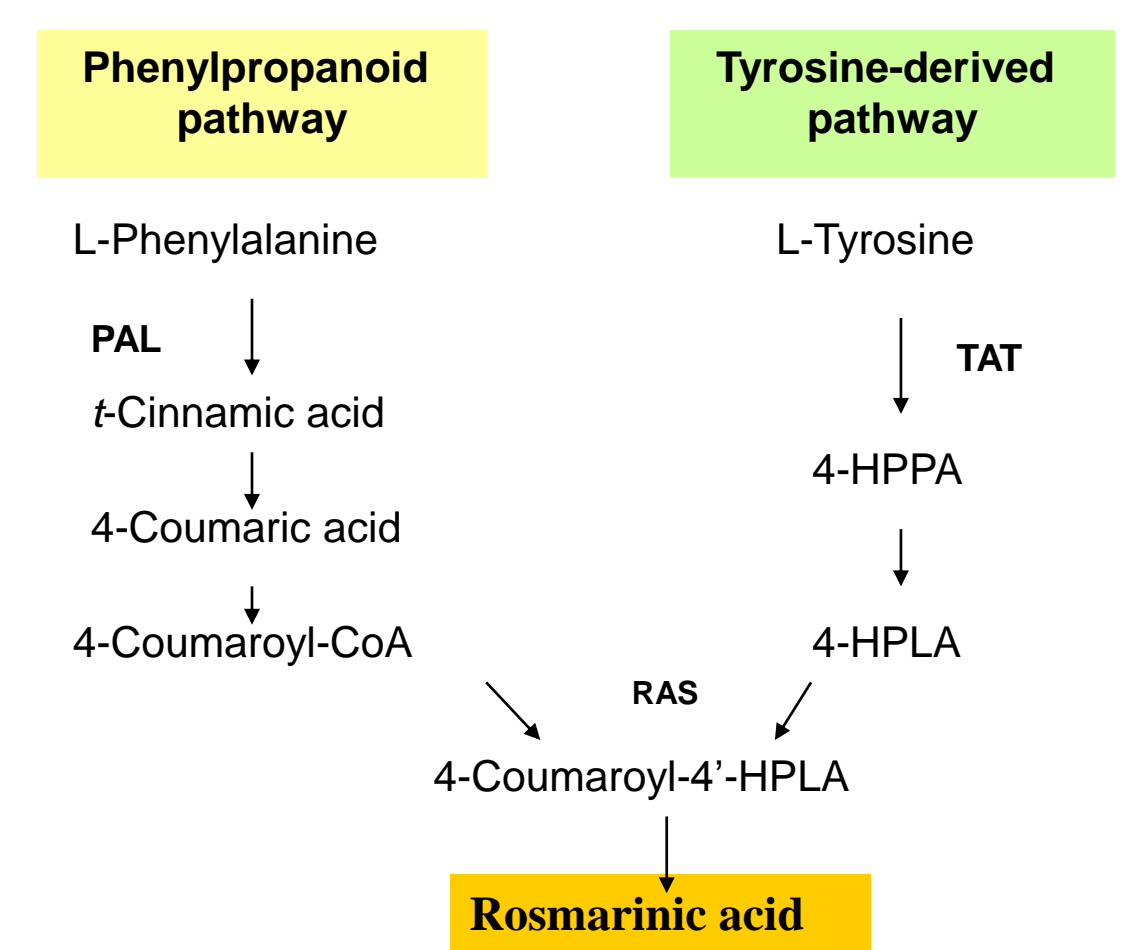
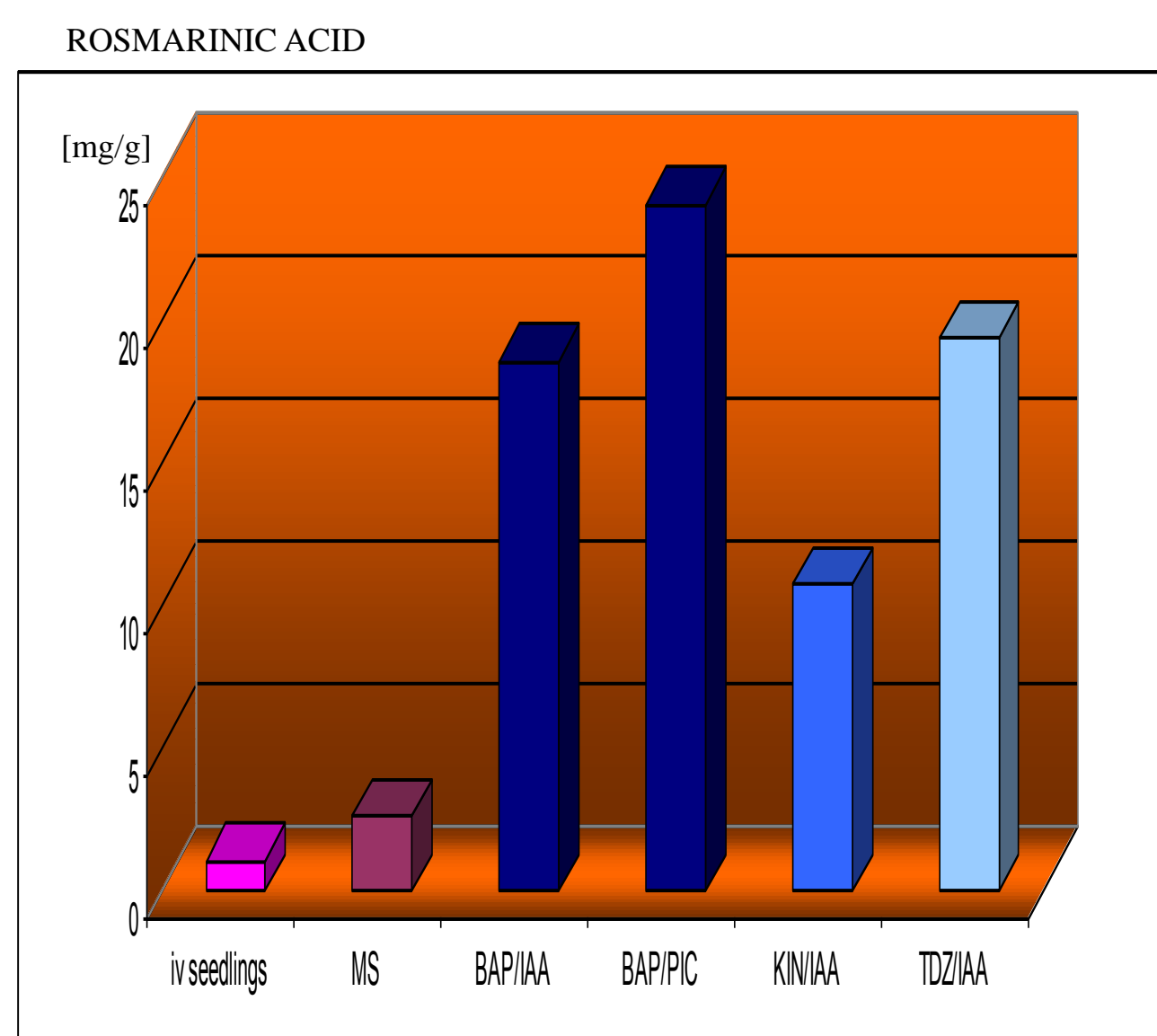
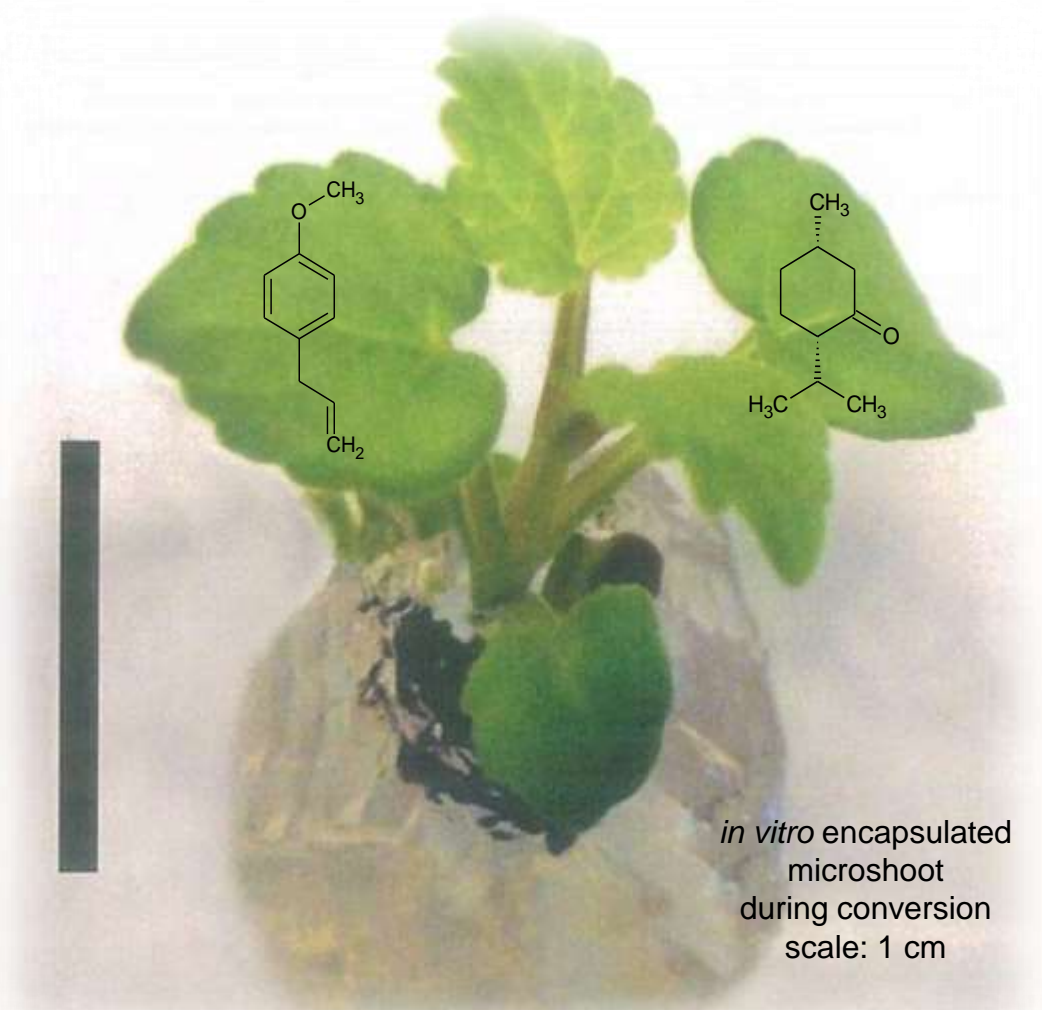


Agastache rugosa (Fish. & Mey.) Kuntze (Lamiaceae), is a traditional Far Eastern (China, Korea, Japan) medicinal plant. The aerial parts of the plant are used and known in Traditional Chinese Medicine.

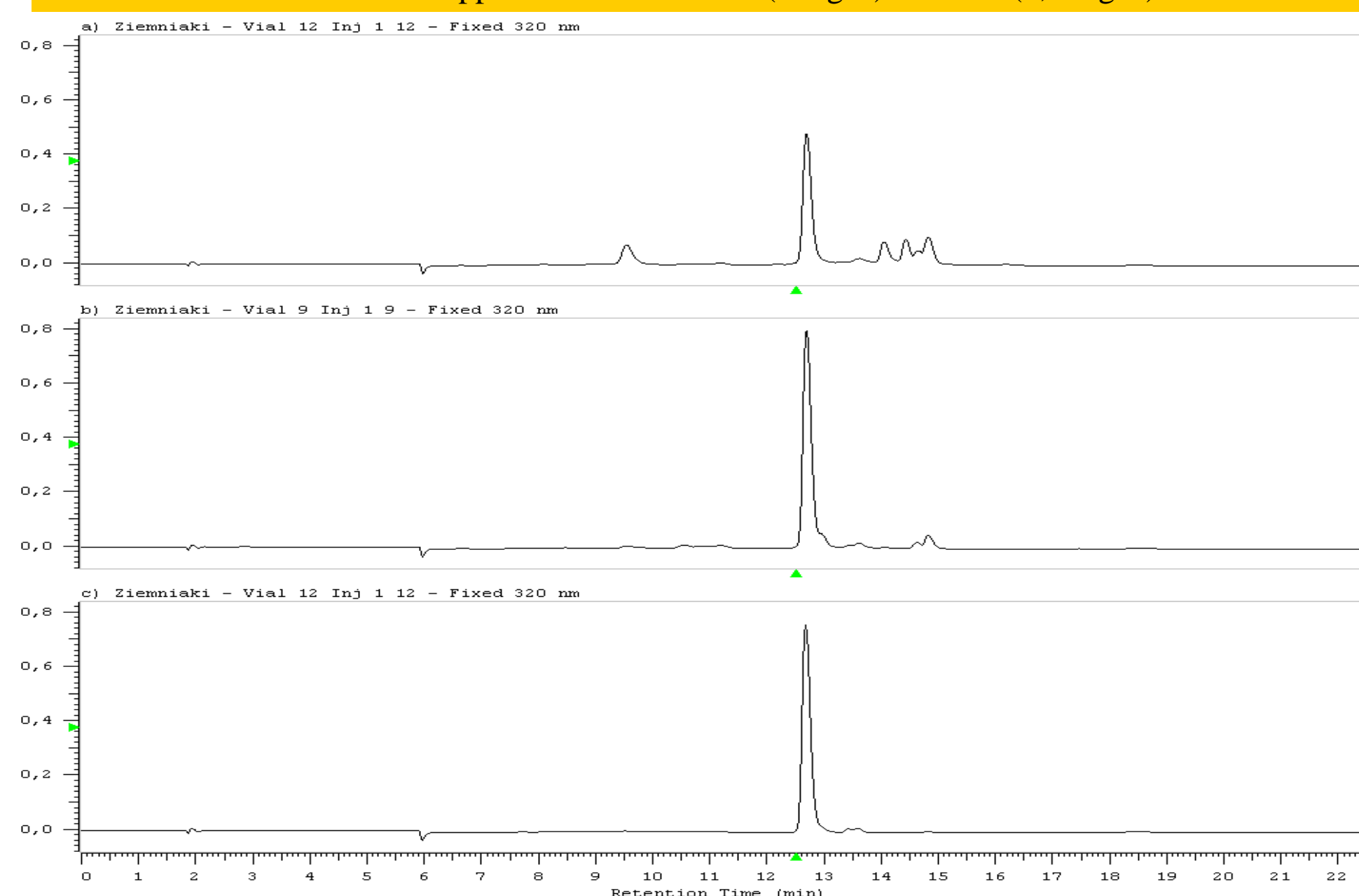
The volatiles are stored in glandular trichomes in aerial parts and are composed mainly of phenylpropanoids (estragole, eugenol, chavicol), monoterpenes (*d*-limonene, α -pinene, *l*-isomenthone, *l*-pulegone), and small amounts of sesquiterpenes (caryophyllene, calamine). The roots of *A. rugosa* contain several oleanane type triterpenes, oxidized abietane diterpenes, and rosmarinic acid as a main polyphenol.

The essential oil has antibacterial, antifungal, and anti-complement activity, attributed to estragole content, that makes up at least half of the oil fraction. Floral essential oil, containing estragol, *d*-limonene, and anisaldehyde is antimutagenic, cytotoxic and immunostimulating.

The present study was focused on the influence of *in vitro* conditions and plant growth regulators on the biosynthetic routes and metabolic profile of non-volatile phenylpropanoids including rosmarinic acid as a major bioactive compound



The HPLC chromatogram of rosmarinic acid content of *in vitro* shoots of *A. rugosa* maintained on MS medium supplemented with BAP (1 mg/L) and PIC (0,1 mg/L)



Establishment of in vitro cultures

Four-week-old *in vitro*-germinated seedlings were used for the establishment of shoot cultures. Shoot tips were excised and individually transferred to a shoot proliferation medium (MS) without plant growth regulators or supplemented with specific concentrations of one of three cytokinins: BAP (benzyladenine), KIN (kinetine), TDZ (thidiazuron), and one of two auxins: IAA (indoleacetic acid) or PIC (picloram).

Determination of Rosmarinic Acid by Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS-MS)

Composition of polyphenols in extract was analyzed using an ACQUITY Ultra Performance LC™ system (UPLC) with binary solvent manager (Waters Corporation, Milford, USA) and a Micromass Q-TOF Micro mass spectrometer (Waters, Manchester, U.K.), equipped with an electrospray ionization (ESI) source operating in negative and positive mode. For instrument control, data acquisition and processing MassLynx™ software (Version 4.1) was used.

Extraction procedure of phytochemicals from in vitro plant material

Agastache rugosa in vitro cultures (0,1 g) were extracted twice with 1 mL methanol and 4 ml 4,5% formic acid. The samples after centrifugation and filtration were directly analyzed by UPLC-DAD-MS-MS.

