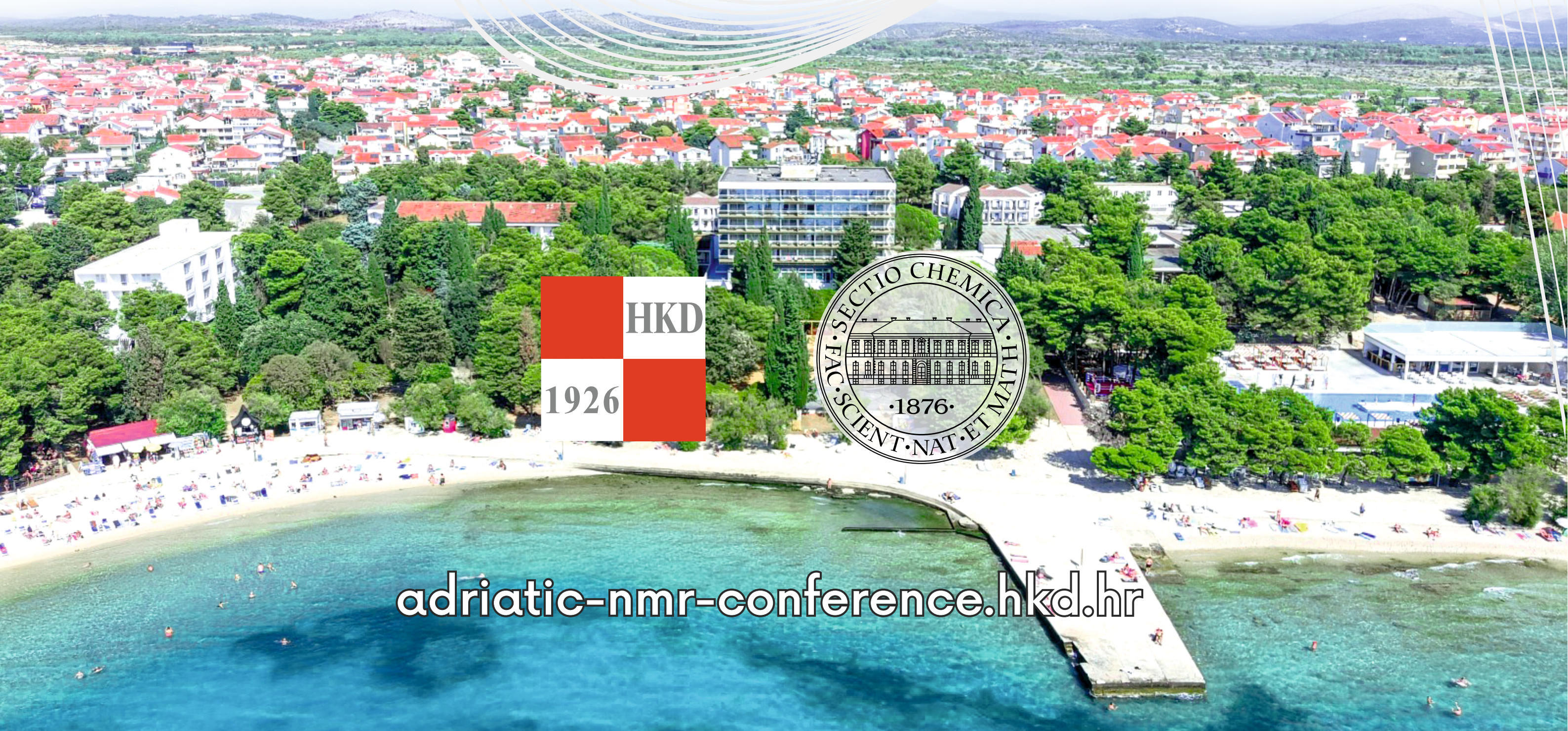
ADRIATIC NMR

18-20 September 2025, Vodice, Croatia

BOOK OF ABSTRACTS



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METABOLOMIC ANALYSIS OF *TRIFOLIUM PANNONICUM* USING NMR SPECTROSCOPY AND CHENOMX: ADVANTAGES AND LIMITATIONS

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Hungarian clover (*Trifolium pannonicum* Jacq.), a previously underutilized forage legume of growing agricultural importance, was investigated for its metabolomic complexity and antioxidant potential.[1] Two genotypes—Panon (a cultivated variety) and a natural Balkan population—were compared to *T. hybridum* (Swedish clover) as a control. A total of 52 samples, collected at the budding stage optimal for forage use, were extracted with solvent mixture (MeOD: buffer KH_2PO_4 in D_2O, v/v = 1:1) and analyzed by ^1H NMR spectroscopy. Multivariate analyses (PCA and OPLS-DA) revealed clear genotype-level separation. Combined 1D and 2D NMR experiments (COSY, HSQC, HMBC), supported by spectral database comparison (Chenomx), led to the identification of 24 metabolites, including amino acids, sugars, and phenolic acids—most notably eucomic and hydroxyeucomic acids. Special attention was given to evaluating the advantages and limitations of the Chenomx platform. Chenomx offers robust tools for metabolite identification and quantification, particularly in complex biological samples. Its capacity to resolve overlapping signals through the utilization of an integrated spectral library facilitates relatively accurate compound profiling in the absence of pure standards.[2] The software's simplicity allows even users without advanced NMR expertise to navigate it effectively. However, its performance strongly depends on spectral quality and database coverage; unidentified compounds and spectral overlap remain major limitations. Moreover, given that the software is optimized for 1D ^1H NMR, its utility in resolving structurally complex metabolites is limited. In this study, the characterization of eucomic and hydroxyeucomic acids in complex mixtures required verification through 2D NMR experiments. While Chenomx enabled initial spin system identification, full structural elucidation was only possible using complementary 2D NMR experiments.

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