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Identifying natural synergist from *Pongamia pinnata* using high-speed counter-current chromatography combined with isobolographic analysis

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For identifying the synergistic compounds from *Pongamia pinnata*, an approach based on high-speed counter-current chromatography (HSCCC) combined with isobolographic analysis was designed to detect the synergistic effects in the complex mixture. In the approach, the complex mixture was considered as the combination of two individual samples for isobolographic analysis: the target compound and the mixture with complete removal of the target compound (subtracted residue). The two samples were prepared by HSCCC, and were used for the calculation of the expected effect of their combination. Using this approach, three compounds representing the major peaks in the HPLC of the brine shrimp toxic extract from P. pinnata (brine shrimp lethality test (BST) LC50 36.5 μg/mL), pinnatin (1), 3,7-Dimethoxy-3,4'-methylenedioxy flavone (2), and karanjin (3), were prepared from the extract, and were tested for their synergistic potency by BST. The two-phase solvent system containing n-hexane-ethyl acetate–MeOH–water (14:7:10:10, v/v/v/v) was selected for the one-step HSCCC separation according to the partition coefficient values (K). The extract was separated into seven fractions (Fr1–7) by HSCCC with a total mass recovery of 96.3%. Fr2, 4, and 6 were the peak fractions corresponding to compounds 3, 2, and 1, respectively. The purities and recoveries of the target compounds after the chromatographic analysis were 95.9%–97.5% and 92.2%–96.1%, respectively. The subtracted residue of each target compound was performed by recombining all HSCCC fractions except the fraction containing the target compound. Isobolographic analysis disclosed a significant synergistic effect between karanjin and its subtracted residue (potency ratio of 0.47), which gave clear evidence that the toxicity of the extract results from synergistic interactions, and karanjin was one of the synergists participating in the interaction. The other two compounds were excluded from the synergism because these two compounds showed additive effects with their subtracted residues.

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Analysis of free fatty acids in olive oils by UPHLC–MS

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A simple, fast, highly efficient and direct method using ultra-performance liquid chromatography coupled to mass spectrometry has been established for the simultaneous separation, identification and quantitation of a few saturated and unsaturated fatty acids in olive oils from various countries. Many methods have already been found in the literature for the analysis of fatty acids. No sample pretreatment techniques were employed such as extraction or derivatization for the analysis of target acids from oil samples, as the oil samples were just diluted, filtered and then directly injected to the instrument. The chromatographic separations of all target fatty acids were achieved on a Hypersil Gold C18 column of particle size 1.9 μm, 50×2.1 mm I.D, while the gradient elution using a binary mobile phase mixture of acetonitrile and water at a flow rate of 1.5 ml/min was adopted for achieving optimum separations. The identification and quantitation of target compounds was accomplished using selected ion reaction monitoring mode. The recoveries of the fatty acids were obtained higher than 89% with good validation parameters; linearity (r2>0.992), detection limit between 0.09 and 0.24 μg/ml, run to run and day to day precisions with percent relative standard deviation lower than 2.4% at both low (1 μg/ml) and medium (10 μg/ml) concentration levels. The total content of fatty acids in each individual oils was found in the range of 472.63–7751.20 μg/ml of olive oil, while oleic acid was found to be the major fatty acid among all analyzed oils with the amount 3785.94 μg/ml (maximum) in Syrian olive oil. The obtained validation parameters confirm that the proposed analytical method is rapid, sensitive, reproducible and simple and it could be applied for the successful evaluation of fatty acids in various oils and other matrices. All the fatty acids were efficiently eluted in a time of less than 8 min with well resolved peaks by employing the proposed method.

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