Optimization of the extraction of secondary metabolites from *Vigna unguiculata* L. Walp. leaves through a DoE approach and evaluation as a functional food

Sartirana C.[1], Guzzetti L.[1], Pioltelli E.[1], Copetta A.[2] and Labra M.[1]
E-mail: c.sartirana1@campus.unimib.it

[1] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, Milan (Italy)
[2] Crea-Research Centre for Vegetable and Ornamental Crops, Sanremo, Italy

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Abstract:
Cowpea (*Vigna unguiculata* (L.) Walp.) is an annual herbaceous plant species belonging to the Fabaceae family. Its importance in Sub-Saharan African regions is determined by its tolerance to biotic and abiotic stresses, such as plant pests and arid climates. Cowpea is mainly used for food as its seeds provide a high protein content and a good micronutrient profile.
In recent years, attention has also moved to other edible parts of the plant: the adoption, use, and consumption of cowpea leaves are rising. In fact, cowpea leaves are used for human health purposes, since they contain many different nutrients such as proteins, vitamins, flavonoids, and microelements. Furthermore, leaves are often considered agricultural waste, thus supporting recirculation and re-valorization of cowpea cultivation.
Therefore, the aim of this work was to create a design of experimental (DoE) that considered the combination of three independent extraction factors (drug-solvent ratio (1:10, 1:20, 1:40 w/v), extraction solvent (EtOH 20%, EtOH 50%, EtOH 80% v/v) and extraction cycles (from 1 to 4)) through the exploitation of an ultrasound-based approach in order to reduce as much as possible the solvent usage and therefore the environmental impact. The evaluated responses were based on the estimation of the total phenol content (Folin-Ciocalteau’s assay) and the total antioxidant activity (DPPH and ABTS assays), highlighting that the optimal extraction conditions were 1:40 w/v, 1:1 v/v ethanol:water coupled with three extraction cycles. The leaves were also subjected to a boiling process simulating the cooking conditions of traditional food African recipes. The results show a net decrease in the phenolic content of boiled leaves. Finally, the optimized hydro-alcoholic extracts were analyzed by UPLC-UV-ESI-QTOF-MS to identify their phytochemical composition and to investigate the differences occurring between the two conditions (raw and cooked) in the metabolic profiles.