Chemical analyses and extraction techniques for quality control of food and dietary supplements

Giuseppe Mannino
Department of Life Sciences and Biological System (dBIOS), Plant Physiology Unit (PPU)
giuseppe.mannino@unito.it
Tutors: Massimo E. Maffei (UniTO) Giuseppe Dedonno (Biosfered)

The main topic of my doctoral thesis is focused on the quality control of food and dietary supplements using advances analytical methods coupled with biomolecular and biochemical techniques. Although food and dietary supplements are strictly regulated, very often the chemical characterization is object of controversial debates. Actually, very often the dietary supplements market is characterized by claims on the content of bioactives present in the raw material that sometimes are far from being reliable. In most of the cases, wrong titrations occur when the identification of the main bioactive molecules is based on simple chemical characterization or on wrong and amateur protocols.

In this context, I focused my thesis on the combination of the genetic and chemical approaches in order to provide an unequivocal identification of raw materials to support identification when the simple evaluation of morphological characteristic is not possible. In the case of pistachio (*Pistacia vera*), spectrophotometric assays (Folin-Ciocalteu, pH jump and DMAC) were combined with either HPLC-DAD-MS/MS or GC-MS-FID analysis in order to investigate the phytochemical diversity within six pistachio verities of commercial interest (*Bronte, Larnaka, Mateur, Mawardi, Kern and Kerman*). Moreover, DNA fingerprinting coupled with RFLP analysis was assessed to determine the differences in non-transcribing regions (ITS and NTS). The chemical and molecular characterization was followed by an evaluation of the biological activity in terms of antioxidative (AOA) and antiproliferative (APA) activities, along with qRT-PCR and enzymatic activities.

Another interesting aspect of the dietary supplement market is the overestimation of bioactive compounds. For example, in the case of *Boswellia* supplements, a concentration of boswellic acids equal to or higher than 70% or AKBA contents of 30% is frequently reported in both *Boswellia sacra* and *Boswellia serrata* gum resin extracts. Due to the importance of these extracts in the treatment of several disease, such as inflammation and osteoarthritis, asthma and age related disorder, the qualitative and quantitative determination of the bioactive boswellic acids is crucial. By the use of HPLC-DAD-MS/MS analytical instrumentation, I provided a solid guideline for the chemical standardization and validation of all dietary supplements containing Boswellia extraccts.

Finally, HPLC-MS/MS technique was instrumental for the identification and quantification of biogenic amines in a collaborative effort with zoologist to evaluate the correlation between the locomotor activity and aggression of *Myrmica* ants in response to Oregano volatile compounds (i.e., thymol and carvacrol). We found that these volatiles affect important biomolecular and biochemical pathway in ants leading to ant behavior changes.

In conclusion, the results of this doctorate period demonstrated that only analytical methods based on gas/liquid chromatography coupled to mass spectrometry allow the precise quantification and identification of bioactive compounds in different plant and animal extracts, and that coupling this methodology with biomolecular and biochemical methods allows obtaining precise information useful for the unequivocal identification of plant species.